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ECOLOGY OF 'CLADOPHORA FRACTA' AND 'CLADOPHORA GLOMERATA'

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Federal Water Pollution Control Administration
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ECOLOGY OF CLADOPHORA FRACTA AND CLADOPHORA GLOMERATA

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For Project WP 00782

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I INTRODUCTION

It has been noted that the high degree of treatment achieved in sewage treatment plants today does not significantly reduce the concentration of the nutrients. Sewage treatment leaves the phosphates and nitrates in the effluent, which is discharged into water courses. Streams and lakes receiving raw wastewater or sewage effluents which have had some degree of treatment have shown significant increases in algal productivity. Nutrients such as phosphorus and nitrogen have been reported as the causes of Cladophora growth in the Great Lakes and many streams.

Cladophora are perennial green algae. They are filamentous and repeatedly branched. Most species require a firm, stable substrate for attachment. Cladophora are distributed throughout the world in both fresh and salt water and occur in unpolluted waters. Cladophora glomerata is the common form occurring in streams and rivers of the North Temperate Zone. Cladophora fracta is the common filamentous alga growing along certain sections of the shoreline of the Great Lakes in both the United States and Canada.

Although Cladophora itself may not be directly regarded as a public health problem, it may create a nuisance and affect the best uses of the water. It has been reported

that certain sections of shoreline along the Great Lakes are covered with Cladophora fracta and each year the affected area increases. Problems caused by Cladophora accumulations in the Great Lakes have been reported from both the United States and Canada. Decomposing Cladophora emit a strong " pigpen " odor, causing economic loss to many riparian citizens, such as recreational and business losses. When large numbers of Cladophora are present, they may impart to water supplies a bad taste, clog screens, interfere with swimming and boating, and reduce fish production. From the Rochester area of Lake Ontario alone, several tons of Cladophora fracta are raked up daily during the summer months.

Cladophora glomerata often form massive growths in streams and rivers. These growths may cause problems such as the depletion of the dissolved oxygen content of the water at night due to their respiration, the development of odor in the process of decomposition, changing of the normal waterway, and the interference with other aquatic life. It has been reported that Cladophora have interfered with the operation of gates in irrigation channels in the South-West.

Cladophora problems are steadily increasing and becoming acute because the rich nutrients from domestic and industrial wastewaters are discharged continuously into

streams and Lake Ontario. Action is required to prevent the death of Lake Ontario. For the past decade, the Ontario Water Resources Commission has been conducting studies on the Cladophora nuisance in Canadia Waters. However, in the United States, little attention has been devoted to study concerning Cladophora.

In recent years both the sanitary engineer and the biologist have been concerned with the study of algae. But most works have been done on the planktonic forms of algae with few efforts devoted to the attached filamentous forms. Since few investigations have been conducted on Cladophora and there is a lack of knowledge about the biology of the alga, this investigation has as an over-all objective the study of the interrelationships between environmental conditions and the growth of Cladophora. The experiments were conducted to: 1) determine the respiration and photosynthetic rates of Cladophora fracta and Cladophora glomerata under natural and laboratory conditions; 2) ascertain the nitrate and phosphate requirements of Cladophora fracta and Cladophora glomerata under laboratory conditions.

These investigations furnish fundamental knowledge concerning the physiological ecology of Cladophora. This information can be used to devise an effective method of controlling excessive growth of Cladophora and can be applied to the study of stream sanitation.

II. LITERATURE REVIEW

A. Cladophora

1. Introduction

Cladophora are algal members of Chlorophyta or the green algae. This genus is usually characterized by filamentous branching, with multinucleate cells. The cells are cylindrical, three to 20 times longer than their width. They are connected end to end. The branches are smaller than the main axis, or at least tapering slightly toward the apices. The cells have thick stratified walls with an inner cellulose layer of an insoluble substance, a chitin-like material. These cells contain a large central vacuole and many pyrenoids. They are multinucleate with the nuclei internal to the chloroplast. Thalli are usually sessile and are attached to the substrate by rhizoidal branching. Most species have an isomorphic alternation of generations. The asexual reproduction is by zoospores while the sexual reproduction is by isogametes produced in apical or subapical, unspecialized cells (Smith, 1933; Prescott, 1951; Tiffany et al., 1952; and Pelczar et al., 1965).

There are numerous species of Cladophora in both fresh and salt water. This genus, Cladophora, includes about 160 species which are not well defined (OWRC, 1959; and Pelczar et al., 1965). They are distributed throughout the world

(Valet, 1960; Oztig, 1962; and Hoek, 1963). Some forms that are permanently attached may exist through the winter in lakes and become wave washed and lose their original appearance, but other species in lakes become free-floating and occur as tangled mats of so-called "Cladophora-balls".

The taxonomy of the genus Cladophora is difficult because of a considerable degree of morphological variation within a species, apparently a result of varying environmental conditions (Prescott, 1951). In recent years, the world's authority on the genus Cladophora, Dr. C. van den Hoek has been investigating the taxonomy of the European representatives of the genus. He uses the following criteria for the taxonomic studies: (1) color, (2) diameter and the length-to-width ratio of the cells, (3) ramification, mode of growth, and thallus organization, (4) insertion of the branches, (5) features of the attachment organ, and (6) mode of reproduction (Hoek, 1964).

It is difficult to culture the filamentous algae because of the lack of knowledge about them. This is true of Cladophora and Prescott (1956) considered it one of the most difficult algae to culture.

In streams and lakes of the North Temperate Zone, which are unpolluted, the genus Cladophora is usually the

principal filamentous alga. In most of the United States and Canada, the species which is predominant in the littoral regions of the Great Lakes is reported to be Cladophora fracta (Dillw.) (OWRC, 1959; McLarty,1960; and Neil, 1962 and 1964), while Cladophora glomerata is the common filamentous alga occuring in rivers and streams (Blum, 1956).

2. Cladophora fracta (Dillw) (Figure 1)

This species forms coarse, light-green masses of irregularly branched filaments with branches which are often curling. The cells are irregularly swollen or clavate, and 60-120 μ in diameter in the main axis. The ratio of their length to diameter is one to three times. The ultimate branches are 20-40 μ in diameter and 3 to 6 times their diameter in length (Prescott, 1951; and Chapman, 1962).

3. Cladophora glomerata (L.) Kuetzing (Figure 2)

The plant mass forms dark green, attached, fluffy or streaming arbuscular thalli, usually in rapidly flowing water, but never in still water. Filaments are successively and regularly branched, usually Y-shaped. The branches are usually crowded in the upper limits. The cells are very slightly attenuated toward the apices of the branches, which are bluntly pointed. The main axis of the cell is 45-150 (mostly 65-125) μ in diameter and 300-1,000 μ

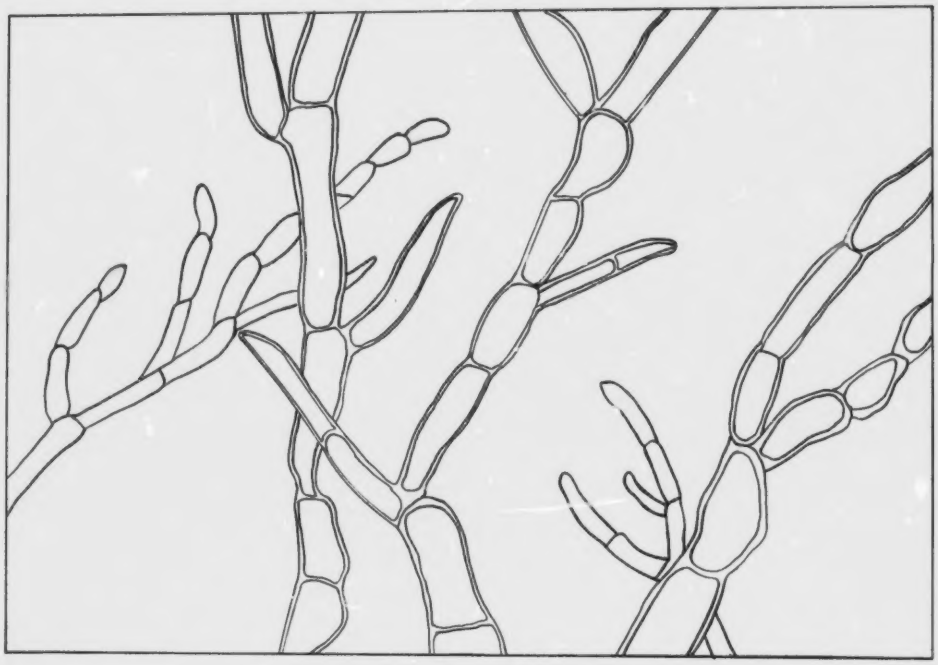

Figure 1. Cladophora fracta. Collected from Lake Ontario, Oswego, New York, (100 X).

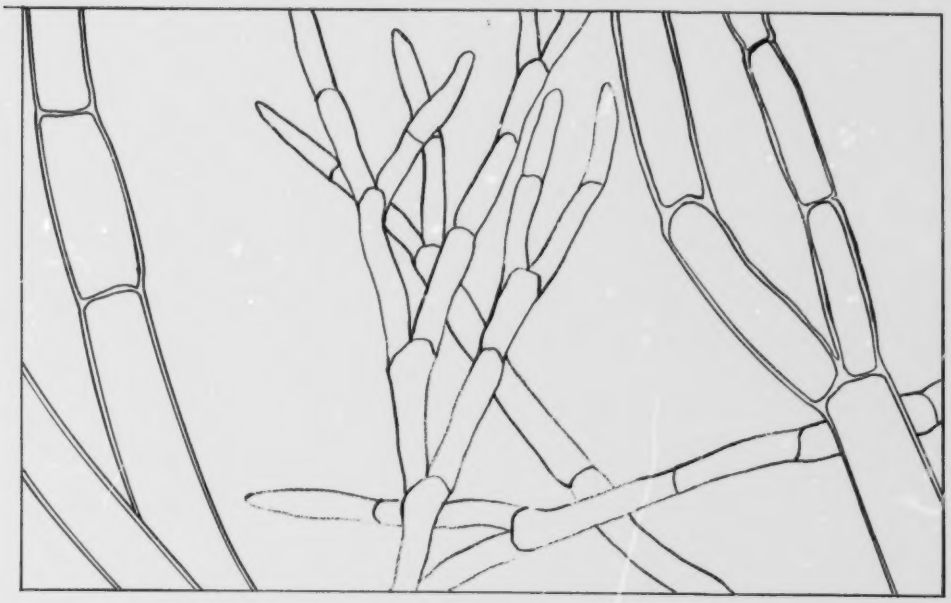

Figure 2. Cladophora glomerata collected from Butternut Creek, near Jamesville, New York.(100 X)

in length. Cells in the branches are 35-50 μ in diameter and 3-6 times the diameter in length (Prescott, 1951; and Tiffany et al. 1952).

4. Cladophora Problem in the Great Lakes

In Canada, since the early nineteen-thirties, health agencies have received complaints about the decomposing algae,malodorous accumulations along the shoreline in parts of Lake Ontario and Lake Erie (Neil and Owen, 1964). For the first time, during the summers of 1957 and 1958, excessive growths of Cladophora were found on rocky out-cropping along the north-westerly shores of Lake Ontario in waters up to and slightly exceeding six feet in depth. In water approximately ten feet in depth the growth of Cladophora was negligible. Fundamental studies on the genus Cladophora and its growth requirements, upon which any control program will ultimately depend, were conducted by the Ontario Water Resources Commission (OWRC). On November 20, 1958, there was a conference on the problems related to Cladophora in Lake Erie and Lake Ontario. It was recognized that the rate of production of Cladophora in the Great Lakes had increased significantly and was a major problem (Berry, 1958).

Chemical algicides were used in 1959 and the growth and distribution of algae created little or no problem in 1960; but the problem arose again in 1961 with Lake Erie,

which is the southernmost and a relatively shallow lake, suffering more than the others (OWRC, 1962).

Cladophora growths first appear in the spring during early May as a fringe at the waterline. During May and June a rapid growth and spread of the algae takes place and by the early part of July all the suitable habitat is covered with filaments up to 15 inches in length when the algae may cease to grow. At this time the filaments are less firmly attached to the bottom and are readily broken by wave action. In late June and early July strong onshore winds scour the algae off the bottom and pile it along the shore. After the removal of the first crop, further growth generally occurs, but the quantity produced is usually less. It appears that the initial period of growth is the most favorable one. It was observed that variations in the pattern of growth and total production occur from year to year in the same lake (Neil, 1962 and 1964).

The growth of Cladophora is generally not excessive, and its presence does not constitute a nuisance. However, excessive growths of these algae may accumulate and disintegrate, thus causing a nuisance. In the Great Lakes, the accumulations range from only a trace to piles three feet deep and forty or fifty feet wide and these may cover extended sections of shoreline. There is no health hazard associated with these accumulations, but when the algae

accumulations occur along the shore, that portion of the algae lying in the warm water begins to decompose. Within two or three days the algae lose their woolly texture, turn black and produce a strong "Pig-pen" odor in the process of decomposition.

The odor and unsightly conditions seriously affect the aesthetic and recreational value of large areas of shoreline. The decomposition of algae often affects the quality of municipal water supplies and frequently clogs intake screens. For example, from December 28, 1959 to January 2, 1960, the water intakes of the British American Oil Company were plugged by masses of Cladophora. It has been reported that those algae masses were healthy and fresh at that time and were carried in by strong easterly winds (OWRC, 1959).

The portion of the algae which has been thrown high enough on the beach to dry, forms a hard, cardboard-like consistency and does not create any odor nuisance. However, a small dipterous fly is attracted to these accumulations of dead Cladophora and may constitute a nuisance by virtue of numbers (Neil, 1962).

What causes the Cladophora problems in the Great Lakes? It is presumed that the quantities of nutrients supplied to the lakes have increased during the past years, such as the rapid growth in the use of commercial fertilizers on farms and in the use of synthetic detergents, together with additions from sewage effluents, both industrial and domestic

wastes.

5. Control of Cladophora in the Great Lakes

The first applications of algicidal chemicals were made on July 28, 1959. Chemicals such as copper sulphate, tribasic copper sulphate, sodium arsenite, Hyamine, and Aqualin were used, and tested for effectiveness. Aqualin proved to be the best (OWRC, 1959). As a consequence, the algal accumulations along the shore,creating nuisance conditions, were at a minimum in 1960.

In 1960, Endothol and TD-47 also were used in a test of algicidal chemicals. McLarty (1960) reported that TD-47 produced a 90 per cent kill in Lake Erie when applied at the rate of 0.5 ppm, while Endothol applied at the rate of 2.0ppm had only a temporary influence on the Cladophora.

In 1962, the testing chemicals were Hydrothol, Aqualin, Hyamine 3500, diaquat, parpquat, copper sulphate, NIA 5625, Phygon, Garlon, and copper sequestrene. These chemicals were used for either laboratory or field tests. Laboratory studies indicated Hydrothol (Penco 47 or TD 47), NIA 3625 and Aqualin to be the most effective. It was shown in field studies that Hydrothol was effective in killing Cladophora in tests made in Lake Erie and Lake Ontario. Aqualin worked well in Lake Erie, but was not sarisfactory in Lake Ontario. Two chemicals were used to control odors associated with decomposing accumulations of algae. Hyamine 2389 provided some temporary

relief. Ozone gave some immediate odor suppression, but the residents objected to the antiseptic smell of the compound (Neil, 1962).

After many years of Cladophora investigations, the Ontario Water Resources Commission concluded that knowledge of the biology of the genus Cladophora is very important in determining the reasons for the excessive growth of Cladophora and to develop a practical means of control. Neil (1964) has given excellent description of the present knowledge of the biology of the genus Cladophora.

6. Studies on the Genus Cladophora

In recent years, studies on algae have been made and are of interest to both the sanitary engineer and the biologist. Most work has been done on the planktonic forms of algae and little on the attached filamentous forms.

The majority of studies on Cladophora and other filamentous algae has been done in countries other than the United States. In England, the ecology of rivers was studied by Butcher (1933 and 1947). He reported that Cladophora glomerata is one important species in streams. The genus Cladophora has become a serious problem in England (Hynes, 1963). Ives (1957) also claimed that the taste and odor problem in drinking water supplies was caused by an actinomycete, Streptomyces, which has a symbiotic relationship

with Cladophora. An excellent study on the cell wall was done by Frei and Preston (1961). The outstanding and extensive investigation of the European representative of the genus Cladophora has been carried out by Dr. van den Hoek. He has published numerous papers on this genus and his 1963 report is the authoritative source (Hoek, 1963).

In Russia, Alfinuv et al. (1961) reported that Cladophora which lives in water with high salt concentration has considerably increased total ash content, as well as calcium and phosphate in the ash. The content of these elements increases approximately one to two times. In samples collected in water with low salt concentration, the amount of thiamine and nicotinamide was four times greater than in samples taken in water with a high salt concentration. In riboflavin content, the reverse relationship was noted.

In France, according to Valet (1960), more than 20 species of Cladophora have been collected in the Sete region, and most are newly identified species. Chihara (1960) studied the life history of the green algae in warm seas around Japan. From the detailed observations on seasonal growth, reproduction and development, it was concluded that the genus Cladophora has an affinity with the Siphonocladales.

Simons (1960) reported on some species of Cladophora from South Africa. Two typical species of the green alga, Cladophora, found in fresh-water lakes of Turkey, are examined from the point of view of morphological character

and ecological conditions. They are found in different places: C. macrognia Kg. in Egridir, and C. callicoma Ktz. in Beysehir (Oztig, 1962). Considerable differences are noted in the mode of ramification and as well as in cell wall and chromatophore structure.

In North America the outstanding current studies on the genus Cladophora have been done by the Ontario Water Resources Commission, Toronto, Canada. A conference was held on November 20, 1958, entitled "Algae Cladophora in Lake Erie and Lake Ontario". At this conference it was agreed that the rate of production of Cladophora in the Great Lakes had significantly increased and was now a major problem. Since that time, reports of "Cladophora Investigation" are being published annually by the Ontario Water Resources Commission or by their workers (Berry, 1958; OWRC, 1959 and 1962; McLarty, 1960; Neil, 1962; Neil and Owen, 1964). The conclusion of these reports is that more ecological studies are necessary to evaluate the role of Cladophora in the Great Lakes and to control it. Studies concerning taxonomy, physiology and morphology are lacking.

The Cladophora problem in the Great Lakes has an international scope. Canada since 1958 has spent considerable time and money on the problem and the United States has just begun work on this species. However, in the past, there have been a few attempts to understand the Cladophora.

Schuette and Hoffman (1920) investigated the chemical compositions of the genus. Blum (1956) made an outstanding study on the ecology of river algae, which described the Cladophora glomerata in detail. In 1958 Wien described Cladophora as a serious nuisance in irrigation waters. He found that Cladophora clogged irrigation installations. Stumm(1960) studied the internal structure of Cladophora seriata. Mason (1962) made a significant contribution to the ecological study of Cladophora glomerata (L.) Kuetzing. Through his studies of five farm ponds, he reported that the algae were found in water when the following conditions existed:

	Min. value	Max. value
Temp.	0.0° C	27.2° C
pH	7.1	10.1
NO_3	0.46 mg/l	19.5 mg/l
PO_4	0.01 mg/l	1.33 mg/l

Turano (1966) studied some ecological aspects of Cladophora glomerata in an artificial stream with controlled environmental conditions. He concluded that the Cladophora glomerata grew better when the pH ranged from 7.5 to 8.0

and the temperature remained between 17° C and 21° C. He also found that the photosynthetic rate increased linearly with phosphate concentration ranging from 0.7 to 5.0 mg/l and was not significantly influenced by nitrate concentration.

Jackson (1966) measured photosynthetic and respiration of Cladophora fracta in Lake Ontario at Oswego, New York and in Henderson Bay, New York during the months of June-July and September-October 1965. He found that the average photosynthetic rate during the early summer months for Lake Ontario at Oswego was 2.63 while that of Henderson Bay was 2.35 µl. O_2/hr./mg ash-free dry weight. The fall months average was 1.27 at Oswego and 0.50 µl. O_2/hr./mg ash-free dry weight for the Henderson Bay area.

7. Photosynthesis in the Algae

Krauss (1959) has presented a review of the major aspects of the photosynthetic mechanism in algae, under the title, "Photosynthesis in the Algae". Discussion on the same topic was made by Palmer (1956), and Oswald and Gotaas (1956). The following are the highlights of these papers:

(a) Carbohydrates

According to Krauss (1959), algae are believed to be the best organisms for the production of carbohydrates and other organic compounds from polluted water. It is

indicated that many uses might be found for the products of algal photosynthesis. At the present time, there is no practical method for the harvesting of algae.

(b) Carbon dioxide

Krauss (1956) pointed out that carbon dioxide utilization in photosynthesis is not limited merely to the initial step. The 0.03 per cent of carbon dioxide, normally found in air, is sufficient to permit an optimum rate of photosynthesis in algae. Algae photosynthesis will change the carbon dioxide concentration which affects in turn the pH of water and carbonates content of the algal cells.

(c) Oxygen

Krauss (1956) stated that algae may release 20 times as much oxygen in photosynthesis as is utilized in metabolism. Growing algae directly in sewage to provide oxygen is relatively new approach to waste-water treatment. This process provides oxygen at the place and time it is needed for biochemical stabilization of the waste organic matter. As stated by Oswald and Gotaas (1957), the growth of one gram of algae is usually accompanied by the production of a minimum of 1.6 grams of molecular oxygen.

(d) Chlorophyll

There are both active and inactive chlorophyll. The

most active chlorophyll tends to be limited to young cells. The measurement of the amount of chlorophyll present in plankton is not necessarily a good indication of the metabolic activities of the algae. Chlorophyll measurement also takes no account of the accessory pigments, presumably influencing photosynthesis, which are present in blue-green algae, diatoms, many flagellates, and other forms (Palmer, 1956).

(e) Light

Light is of first importance as a factor for photosynthesis. A dark period is also required. Theoretically, the optimum relationship is an alternation of 0.001 second of a light period with 0.01 second of darkness (Krauss, 1956). Light intensity, quality, and duration influence the rate of photosynthesis. Oswald and Gataas (1956) indicated that daylight of average intensity of 2000 to 3000 f-c will penetrate approximately 30 centimeters into a culture of algae containing 150 ppm of algal cell material.

(f) Nutrition

In recent years, more attention has been paid to other facets of algae metabolism and especially to their nutrition. Nutrition plays a primary role in the efficiency of photosynthesis. Table 1 (Krauss, 1956) shows the chemical com-

TABLE 1.

ELEMENTAL COMPOSITION OF *CHLORELLA* AND *SCENEDESMUS* (Krauss, 1956)

Element	Dry Weight %
For *Chlorella*	
Carbon	51.4 - 72.6
Hydrogen	7.0 - 10.9
Oxygen	28.5 - 11.6
For *Scenedesmus*	
Nitrogen	7.7 - 2.2
Phosphorus	2.0 - 1.0
Sulfur	0.39 - 0.28
Magnesium	0.80 - 0.36
Potassium	1.62 - 0.85
Calcium	0.08 - 0.005
Iron	0.55 - 0.040
Zinc	0.005 - 0.0006
Copper	0.004 - 0.001
Cobalt	0.0003 - 0.00003
Manganese	0.01 - 0.002

position of Chlorella with respect to carbon, hydrogen, and oxygen. This table also gives the chemical composition of Scenedesmus with respect to other elements. In addition to the elements listed, molybdenum and vanadium are also required for growth. A deficiency in any one of the elements listed may alter metabolism and affect not only the growth rate, but the nature of the organic compounds being produced. Algal cells that are deficient in nutrients are unable to utilize light as efficiently as cells having unlimited access to nutrients.

(g) Temperature

The effect of temperature on photosynthesis in the algae has not been given the critical study it deserves. The initial photochemical reaction is not temperature-sensitive. Optimum temperature range for photosynthesis varies with the species of algae. Some can survive in cold weather; but on the other hand, some algae can grow in hot springs at elevated temperatures, as high as 89° C.

8. Environmental Requirements of Cladophora

It is believed that knowledge of the ecology of Cladophora is most important to the development of control methods in the Great Lakes. Unfortunately, very little has been done on the environmental requirements of the species

of Cladophora. As Neil and Owen (1964) indicated,their attempts to grow the species in the laboratory have been unsuccessful. They did not conduct laboratory culture experiments. Therefore, they studied the physical, biological and chemical, factors on the Great Lakes.

(a) Physical requirements

Four of the most important physical requirements which affect the ecology of Cladophora are water movement, substrate, water temperature, and solar radiation.

1) Water movement

According to Butcher (1933), the current is the primary factor which governs the distribution of the larger plants or macrophytes in running water. The distribution of Cladophora glomerata is most often in the rapid waters of a brook, occupying the crests of the shallows and almost entirely absent from the pools of the same stream (Blum, 1956). Blum (1963) also described that the influence of water current on the algae is significant because nutrients are brought to the algae by the current and the current might carry away harmful substances from the algae. Cladophora fracta is established first in niches where a maximum water movement would be expected. Growth often occurs at the waterline along piers and seawalls in areas which generally do not

support a growth on the bottom. The algae are restricted to fissures and cracks in the bedrock which might be expected to funnel water and provide local turbulence. This is particularly evident in areas considered to be marginal for growth with respect to the fertility of the water (Neil and Owen, 1964).

Currents in the Great Lakes vary from zero to 25 feet per minute (OWRC, 1959). Neil and Owen (1964) concluded that the current is more significant than wave action in governing algal population, and the production of Cladophora will be greater under particularly favorable conditions of flow, even though the fertility of the water is relatively low.

2) Substrate

Cladophora glomerata depends upon the size, nature and stability of substrate found in lotic environments. Cladophora requires a stable, firm substrate for attachment. The algae are almost never found on a smooth substrate, such as clay or silt, but must have fairly large size stones, at least an inch or two in diameter, on which to attach. Neil and Owen (1964) stated that Cladophora in the Great Lakes grows most abundantly on coarse gravel, boulders and bedrock as well as on wood, iron, fiberglass, concrete and painted surfaces.

3) Water temperature

Cladophora has been found to grow within a wide range of temperatures. Cladophora glomerata has been observed at temperatures ranging from 0°C to 27°C (Mason, 1962). Blum (1956) reported that this alga survived throughout the winter. And he also pointed out that the alga has poor growth in warmer streams during mid-summer and probably is sensitive to temperatures higher than 25°C.

The most extensive and rapid growth of Cladophora fracta in the Great Lakes occurs in late June and early July before the water temperature reaches 21°C. The more restricted growth is observed in August and September (OWRC, 1959). It is not known whether this condition is a direct result of water temperature or whether the availability of essential nutrients also becomes a limitation at that time.

4) Light

Light is the primary requirement for photosynthesis. All investigators agree to the need of light,but they do not agree on the intensity of light needed. Tiffany (1958) indicated that Cladophora occurred in Lake Constance at depths to 30 meters and grew in rivers at from seven to 25 meters and needed only a small amount of light. In studies by Mason (1962), the amount of light did not limit the abundance of the Cladophora glomerata in ponds observed, but some evidence suggested that a decrease in

amount of light initiated akinete and zoospore formation.

In Lake Erie, significant growth of Cladophora does not occur below 13 feet and the production is decreased considerably below eight feet. In the Toronto-Hamilton region of Lake Ontario, where the waters are less turbid, the alga grows to a depth of 25 feet and is considerably reduced in depth greater than 12 feet. The alga grows to 45 feet along the shore of Prince Edward County (Neil and Owen, 1964).

Blum (1956) reported that Cladophora grew adversely the reduced light, but could survive in the dark for 12 weeks. He concluded that high light intensity is necessary for abundant growth.

McMillan and Verduin (1953) indicated that, under nearly natural conditions, photosynthetic rates in optimal light absorbed 33 μ moles of carbon dioxide per hour per gram (wet) of Cladophora glomerata at a temperature of about 17°C. They concluded that low light intensities were inefficient for photosynthesis.

(b) Biological relationships

No competition exists between Cladophora and higher aquatic plants. However, competition for nutrients undoubtedly occurs between Cladophora and planktonic algae. Epiphytes, predominantly diatoms, are often numerous on the Cladophora

cells which are entirely covered and usually dead.

No animal forms are known to utilize Cladophora directly for food. In the Great Lakes, Scuds (Gammarus spp.) grow abundantly in the latter part of the summer, but whether they are feeding on the epiphytes or on the Cladophora is not understood. Fish native to the various waters are seen regularly in the beds, and young fish utilize the algae for cover (Neil and Owen, 1964).

(c) Chemical requirements

Chemical requirements of Cladophora will be discussed separately as pH, dissolved oxygen, biochemical oxygen demand (BOD), and nutrients.

1) pH

In general, Cladophora is restricted to hard waters and is reported to be indicative of high pH (Prescott, 1951). Cladophora glomerata grew in ponds with pH of 7.1 and 10.1. Thus, Mason (1962) concluded pH was not directly related to C. glomerata productivity. Blum (1956) also reported that algae would tolerate high pH.

Studies by Hoek (1963) and Turano (1966) show that best results were obtained in cultures where the pH range was 7.5 to 7.7 and 7.5 to 8.0, respectively. Hoek (1963) also stated that Cladophora glomerata does not tolerate pH

less than 7.1 and needs a high pH from 7.5 to 8.5 for optimum condition in nature.

The pH of the Great Lakes water generally lies within the range suitable for optimum growth of Cladophora fracta and is sufficiently constant to exert very little influence on growth (Neil and Owen, 1964).

2) Dissolved oxygen

The dissolved oxygen content of the water plays an important role in the ecology of Cladophora. The alga is found only in waters of high dissolved oxygen content. It occurs most in the rapid running water, in the riffles of a stream where dissolved oxygen is high.

Oxygen can be produced by algal photosynthesis. This is one of the means of reaeration in ponds, lakes, or streams. It is believed that the saturation or supersaturation of dissolved oxygen content in water is due to algal photosynthesis. For example, one gram of Cladophora fracta produces 2.8 - 15.36 milligrams of oxygen per hour (Vertebnaya, 1961).

3) BOD

It is understood that Cladophora grows better where organic matter is high, but the presence of algae is not a good indicator of water pollution by organic matters.

Stated by Hynes (1963), abundant growth of Cladophora glomerata is found primarily where the bio-chemical oxygen demand is falling rapidly and growths do not diminish as the BOD falls to quite low values.

4) Nutrients

The elements essential for the growth of algae are the same as those necessary for the growth of higher plants. These would include the macronutrients, nitrogen, phosphorus, potassium, calcium, magnesium, and sulfur, as well as the micronutrients, iron,manganese, copper, zinc, boron, molybdenum and chlorine. Table 2 shows the organic and inorganic constituents of Cladophora in Lake Mendota, Wisconsin (Schuette and Hoffman, 1920).

If the physical requirement is favourable for growth of algae, adequate nutrients are quite necessary, such as nitrogen, and phosphorus. Cladophora grows in a wide range of mineral nutrient concentrations.

In the Great Lakes, the quantities of nutrients supplied to the lakes have increased during the past years. This so-called "artificial enrichment" was supplied by sewer outfalls, by industrial effluents, by the drainage of fertilizers from farming areas. Based on the physical factors and adequate fertility of the Great Lakes, it is sufficient to support Cladophora growths.

The relation of this alga to nitrogen and phosphorus will be discussed in detail in the next section.

TABLE 2.

THE CHEMICAL COMPOSITION OF CLADOPHORA IN LAKE MENDOTA, WISCONSIN (Schuette and Hoffman, 1920)

Constituent	Dry Weight, per cent
Crude protein (N x 6.25)	18.19
Ether extract	2.00
Crude fiber	17.33
Pentosans	9.10
Nitrogen-free extract	26.85
Ash	26.53
Silica (SiO_2)	7.08
Chlorides (Cl)	0.14
Sulphates (SO_4)	0.64
Phosphorus pentoxide (P_2O_5)	0.32
Iron oxide (Fe_2O_3)	0.49
Aluminum oxide (Al_2O_3)	1.30
Manganese oxide (MgO)	1.62
Sodium Oxide (Na_2O)	not determined
Potassium oxide (K_2O)	not determined

B. Effects of Nitrogen and Phosphorus on Algae

1. Sources of Nitrogen and Phosphorus in Waters

Nitrogen and phosphorus in surface waters are derived from a variety of sources. These include natural sources, decaying organic matter, surface runoff, fixation by microorganisms in soil and water, and organic wastes. They will be discussed separately in the following.

(a) Natural sources

The sources include precipitation, ground water, and soil and rock. Nitrogen compounds are both oxidized and reduced by organisms. Some nitrogen compounds are absorbed on clay. The theoretical end product in water and the compound probably most often determined is nitrate. The concentrations of nitrogen compounds range from 0.0 to 100 ppm in surface water and from 0.0 to 1000 ppm in ground water (Feth, 1966). Seasonal variations occur.

(b) Decaying organic matter

As aquatic organisms die, they settle to the bottom or float on the surface of the water where they are decomposed by bacteria. Various elements, including nitrogen and phosphorus, are released. As a result of bacterial activity, the water becomes depleted of oxygen. In Tucker's (1957) study, a lake that was devoid of oxygen at the bottom and

supposedly should have shown increasing nitrogen and phosphorus contents, showed none until eight weeks after the oxygen had disappeared. Dead bodies of animals and plants also contribute a large amount of nitrogen and phosphorus in soils and in waters.

(c) Surface runoff

Bennett (1939) reported that phosphorus in surface soils of the United States carried to surface waters may be in the single orthophosphate form or as soluble hydrolyzable phosphate, or it may be absorbed on clay particles (Engelbrecht and Morgan, 1960). Silvey (1953) reported that clays act as ion exchange compounds and that certain phosphates in calcium bearing soils are converted to calcium phosphate and remain in the humus or the sub-soil until the pH closely approaches 7.0, at which time the soluble phosphate would be eluted by runoff, percolation, and subsurface seepage.

The use of commercial fertilizers and pesticides increases the phosphate content in soil significantly. This will be carried into the surface water by surface runoff. The same is true for nitrogen compounds existing in soils.

Studies by Engelbrecht and Morgan (1961), indicated the importance of land drainage as a source of orthophosphate, hydrolyzable phosphate, and organic phosphate in streams which drain highly cultivated lands.

(d) Fixation by micro-organisms in soil and water

The initial source of nitrogen in the biosphere is molecular nitrogen from the atmosphere and the nitrogen and nitrogen compounds we find in the aquatic environment are the result of influx, fixing and cycling.

Nitrogen is an important component of the atmosphere. By volume it accounts for about 78 per cent of the gaseous environment. Some combined nitrogen is added by volcanic action and a minor contribution is made by smoke and dust (Hutchinson,1957). However, the aquatic complex depends upon the "fixing" of atmospheric nitrogen. This fixation may occur in the atmosphere through electrical or photochemical processes, with subsequent delivery by rain, or through surface solution, but the activity of bacteria and algae are of far greater significance.

According to Umbreit (1962), there are relatively few organism capable of carrying out nitrogen fixation; they are mostly heterotrophic; some are photosynthetic, and there are both anaerobic and aerobic forms .

(e) Organic wastes

Waste waters are thought to be major sources of nitrogen and phosphorus. These are agricultural, domestic,and industrial wastes. Oswald (1960) estimated that approximately 20 million tons of organic wastes annually must be deposited

directly or indirectly into natural waters. He also points out that the quantity of these wastes is increasing because of (1) the population increase in the country as well as (2) the increased use of water for recreation.

The use of detergents has increased rapidly. Most of the synthetic detergents contain phosphates. The phosphorus content of sanitary sewage has increased in some instances to 100 mg/l or more. Many of these syndets are not readily subject to biological degradation and pass through wastewater treatment plants relatively unchanged. As well as detergents, however, a number of new synthetic organic fertilizers contain phosphorus, while the increased use by farmers of superphosphates has been significant.

2. Growth Characteristics of Algae

The growth and metabolism of green plants require the availability of from 15 to 20 elements. Macronutrients and micronutrients are listed in Table 3 (Eyster, 1964). Some of the elements vary, depending on whether they are utilized by higher plants or by green or blue-green algae. Plants require mineral elements for various functions. The macronutrients are used commonly as building materials, while the micronutrients are generally metal constituents of enzymes which are involved in biochemical reactions (McElroy and Nason, 1954).

TABLE 3.

ELEMENTS REQUIRED BY GREEN PLANTS
(after Eyster, 1964)

Macronutrients (10^{-2} - 10^{-4}M)	Micronutrients (10^{-5}M and less)
C, H, O, N	Fe, Mn, Cu, Zn
P, S, K, Mg	Mo, V, B
Ca*	Cl, Co, Si
Na†	

*Except for algae, where it is a micronutrient.

†For blue-green algae.

Nitrogen and phosphorus play an important role in the aquatic ecosystem, because the synthesis and maintenance of protein is dependent on the availability of the nitrogenous materials. Tucker (1957) stated that (1) phosphorus is an important element in the protein molecule, (2) it plays an important role in the energy metabolism of all living things, and (3) it occurs in lakes, but in very minute quantities.

There are numerous environmental conditions which cause the "bloom" of algae. Such environment requirements are not

solely nutritional. As pointed out by Krauss (1960), attention must be focussed on the physiology of the algae, especially those which play a major role in determining the aquatic community and reciprocally affect the quality of the water that supports them.

Karanik (1964) made a comprehensive literature survey on nutrients and algae. He discussed in detail the following topics; (1) metabolism of algae, (2) algal growth effects, (3) relationship between sewage contamination and fertilizer runoff and algal blooms, (4) nutrient removal by conventional sewage treatment and microorganisms, (5) nutrient removal by alum coagulation and lime precipitation, (6) algae control methods, such as recalcination of lime sludges, (7) use of algae in removing phosphorus from domestic sewage, and (8) three predominant algae found to grow at constant light and constant temperature. Some of these aspects stressed by the author will be discussed.

(a) Metabolism of algae

1) Nitrogen

Many factors account for algal metabolism. These are nutrients, light, oxygen, temperature, and pH. Many investigators agree that nitrogen and phosphorus are limiting factors for algal metabolism (Lazaroff, 1952; Rao, 1953; Ohle, 1955; and Neil, 1957). However, only nitrogen and

phosphorus as they are related to algae will be discussed. Molecular nitrogen in the water is used by some bacteria, fungi, and blue-green algae that have capacities to link this inert free nitrogen with other materials, forming compounds that are in turn assimilated by other plants (Coker, 1954).

Some ammonia and ammonia salts are used directly by certain algae and some are oxidized by nitrifying bacteria, in aerobic conditions, in the production of nitrites. Still other organisms aid in oxidizing these nitrites in forming nitrates. The nitrates in turn are widely used by green plants, or they are reduced by denitrifying bacteria to nitrites, and nitrites to ammonia or free nitrogen, under anaerobic conditions.

Studies by Flaigg and Reid (1954) showed no difference in utilization of ammonia, nitrite, or nitrate by algae. However, Gotaas and Oswald (1957) found that the species of algae which are effective in photosynthetic oxygen production utilize ammonia as the major source of nitrogen.

Many algae are able to fix nitrogen from the atmosphere. Sawyer (1961) concluded that phosphorus is the key element in the stimulation of nitrogen fixation. Gerloff and Skoog (1957) also found that Microcystis aeruginosa have a rather high phosphorus requirement for fixing nitrogen. The trace elements from the sewage are thought to stimulate the nitrogen

fixation of Anabaena (Dugdale and Neess, 1961). In studies on Nostoc muscorum, Lazaroff (1952) found that the alga is able to fix nitrogen in the dark on a medium of either glucose or sucrose. He also concluded that nitrogen-fixing was inhibited by the presence of nitrates and ammonium salts. It is thought that this alga uses calcium carbonate autotrophically.

2) Phosphorus

The active element phosphorus is not found free in nature, but in the form of phosphates in several materials. The phosphates may appear in the form of ortho-, meta-, and pyrophosphoric acids, or anhydrous salts in which the hydrogen of the acid is replaced partially by metals. The water solution of such a salt will contain primarily $H_2PO^-_4$ or HPO^{-2}_4, depending on the pH of the solution. All sodium, potassium and ammonium phosphates are soluble, but of the calcium salts, only monocalcium phosphate is soluble. Most magnesium phosphates are only slightly soluble. In water the meta- and pyro-forms tend to change to the more stable ortho-state, which is referred to as phosphoric acid. Atkin (1923) was the first to conclude that phosphorus might be a limiting factor in the production of phytoplankton.

Juday, et al. (1928 and 1931) found no evidence that phosphorus was a limiting factor of phytoplankton populations.

In 1939, Prescott reported that he has found positive correlations between phosphorus content and productivity of plankton in Iowa lakes. The difference of opinion, which is not uncommon on this subject, may be due to the different forms of phosphorus that were being determined.

Sawyer (1947) reported that the soluble phosphorus content of water may be a factor in limiting the rate of biological activity and in determining the nature of the growths when the concentration drops below 0.01 ppm. Phosphorus as a limiting factor for algal growth has been reported by many investigators (Owen, 1953; Gotaas and Oswald, 1957; and Shapiro, 1962).

Ludwig et al. (1951) found the phosphorus requirement to be greatest for the youngest and most rapid growing cells, indicating that phosphorus utilization is closely associated with photosynthesis. This indicated that phosphorus may be limiting for retention times greater than seven days. Lund (1950) reports that it is known that some algae store phosphorus in excess of that immediately required even when the concentration in the water is very low. In 1963, Mckee and Wolf also noted that Microcystis can store nitrogen and phosphorus in excess of its needs. The same results were concluded by Maloney (1966).

Can algae utilize phosphorus in insoluble form? According to Tucker (1957), Harvey (1937) has evidence of marine diatoms

making use of suspended ferric compound. This would indicate a possibility that other phytoplankton might also utilize it. Chu (1946) pointed out that pyrophosphate cannot be utilized as a source of phosphorus as effectively as orthophosphate by the organisms cultured.

3) Nutrients concentration and the N:P ratio

Nutrient concentrations necessary to produce algal growths will vary, depending on the concentrations of other substances, the water temperature, light and the rate at which nutrients can be supplied, once growth commences (Sylvester, 1960). However, what concentrations of nitrogen and phosphorus will bring a nuisance of algal growth is a matter for conjecture. These values may be very low. By a study of Wisconsin lakes, Sawyer (1947) reported that large phytoplankton blooms may be supported when phosphorus concentrations were 0.01 ppm or above as phosphates and nitrogen concentrations were above 0.3 ppm as nitrates.

It appears that very low phosphorus concentrations likely to be present in natural sources can support substantial algal blooms in natural waters. Nuisance algal blooms were observed in Green Lake, Seattle, when nitrate nitrogen values were generally above 0.2 ppm and soluble phosphorus was greater than 0.01 ppm (Sylvester, 1960). These blooms usually stopped when soluble phosphorus dropped to zero.

Chu (1943) reported that optimum growth of the organisms studied can be obtained in nitrate nitrogen levels of 0.9 to 3.5 ppm and phosphorus values of 0.09 to 1.8 ppm, while a limiting effect on all of them will occur at nitrogen concentrations of 0.1 ppm or less and at phosphorus concentrations of 0.009 ppm or less. Marked inhibition was observed at concentrations of nitrogen or phosphorus of 45 ppm or more.

Sawyer (1952) determined the relationship between the production of algal blooms and the N-to-P ratio. He found that N-to-P ratios of 30:1 were optimal for some algae while for others 15-18:1 ratios were required. He also suggested 0.01 mg/l of inorganic phosphorus as the maximum concentration that can be permitted without danger of supporting undesirable algal growth. Gerloff et al.(1950) also derived an N-to-P ratio of 30:1 as optimum for the growth of a particular species of blue-green algae. Therefore, domestic sewage which contains N-to-P ratio of about 8:1 (15-35 ppm:2-4 ppm) is undoubtedly rich in phosphorus. On the other hand, Abbott (1967) concluded that nitrate and phosphate consumption occur independently, and there is neither inhibition nor enhancement of one by the other.

4) Nutrition deficiency

In studying Green Lake, Fayetteville, New York, a dichothermic and meromictic lake, Jackson and Dence (1958) wondered why little or no planktonic algae existed in it.

Temperature conditions are favorable and sunlight is more than adequate as well as an abundance of free carbon dioxide. They speculated that some factor was lacking or inhibiting photosynthesis in the lake. There may be a lack of nitrates or phosphates.

Chu (1943) contended that the nitrogen concentration determines to a large extent the amount of chlorophyll formed. A yellowing or whitening effect was always observed in nitrogen deficient cultures and a yellowing effect occurred in phosphorus deficient cultures.

(b) Algal blooms

Many surface water algae, under favorable environmental conditions, have the ability to accumulate and to form loose, visible aggregations called "blooms", which may cover very large areas of lakes, ponds, reservoirs, or even streams. The blue-green algae, especially, may form blooms and distribute throughout the water during periods of warm and calm weather. In relation to the algal blooms, the filamentous green algae, such as Cladophora, Spirogyra, Zygnema, and Oedogonium may form "mats" or "blankets" to cover large areas of water (Palmer, 1962). Such algal growth are detrimental; they may kill fish, animals, and even man, as well as cause filtration problems, tastes and odors, recreational losses, and ecosystem imbalances. Palmer (1962) gave many examples of algal

problems in treatment plants, rivers, lakes, and reservoirs of the United States, while the algal problems of Europe were discussed in great detail by Skulberg (1962).

(c) Economic aspects

The ill effects of algal blooms have been mentioned above. However, algae play a small but important part in the direct economy of many countries. Four major products are derived commercially from algae. They are: agar, carrageenin, alginic acid and diatomite (Round, 1965). There are so many other benefits from algal growth that it is impossible to estimate its full economic importance. Algae have the ability to produce oxygen while utilizing organic matter in waters. They are part of the food chain leading to crustacea and fish. They may also serve as food for human beings.

Both ill effects and benefits from algal growth interest sanitary engineers. Therefore, many attempts are being made to use the algae for both waste treatment and water purification processes. Unfortunately, these attempts are not yet in a practical stage.

Among these investigators, Bogan et al.(1960) used algae to remove phosphorus from sewage. Bartsch (1961) used Chlorella as a source of oxygen in trickling filters for waste treatment. Many studies have been made at the University of California by Oswald and Gotaas. They studied ecological control in waste

stabilization ponds; their results are very successful. Gates and Borchardt (1964) studied nitrogen and phosphorus extraction from domestic wastewater treatment plant effluents by Chlorella. Removal of algal nutrients from waste waters is one method of ecological control. Such a study has been done at Syracuse University by Rand and Nemerow (1965).

III. EXPERIMENTAL

A. Experimental Design

A Bronwill Warburg Apparatus was employed for measuring respiration and photosynthetic rates of Cladophora. An artificial stream was also used for culturing of Cladophora. This equipment will be described in detail in the following paragraphs.

1. Bronwill Refrigerated Photosynthetic Warburg Apparatus

The Braun's Warburg Apparatus Model "VL 85" (Figure 3) with Bronwill reaction vessels No. 30-35 and Bronwill No. 5-22 double capillary differential manometer (Figure 4) were used in these experiments. This instrument was obtained with funds from the United States Public Health Service grant WP-68 awarded to Dr. D. F. Jackson.

The Bronwill Warburg Apparatus consists mainly of five parts. They are the thermostatic bath, the thermoregulator, the shaking motor, the light bulbs, and manometers with vessels. The thermostatic bath is filled with approximately 10 liters of distilled water. In addition, 150 ml. of 1.5 per cent sodium chloride solution is added to give the water sufficient conductivity for operation of the heating and refrigeration electrodes. The temperature of the thermostatic bath ranges from 0°C to 60°C. ..d is also included and can

Figure 3. The Bronwill Refrigerated Photosynthetic Warburg Apparatus

Figure 4. Bronwill reaction vessels and Bronwill double capillary differential manometer.

be adjusted to six different speeds by changing the transmission V-belts. The light source is supplied by fourteen 40-watt, frosted, incandescent light bulbs, each of which was located directly under and seven centimeters away from the reaction vessel.

2. Artificial Stream

The artificial stream was constructed and used by Turano (1966). The basic frame was constructed from lumber and plywood. The stream channel is a regular trapezoid in shape with nine and 26 inches of two legs, eight inches in depth, and 16 feet in length. The surface of the upper channel was waterproofed by covering with fiber glass and Marine Bond polyester resin. The resin was manufactured by Marine Bond, Syracuse, New York. There is an overflow weir at the end of the channel to regulate the water level in the channel from one to eight inches. The inlet tower (2.0 x 2.0 x 2.5 feet) has an adjustable sluice gate to aid in the regulation of flow.

The bottom reservoir, 15 x 19 inches in cross-section and 18 feet in length, beneath the stream channel was lined with a 0.6 mm. thickness of polyethylene plastic sheet. A water pump was employed to bring the water from the bottom reservoir to the upper channel. This water pump was manufactured by Ingersoll-Rand with a pumping capacity of 150 gallons per minute. A cooling system was also used in the

bottom reservoir. The compressor was manufactured by Bendix-Westinghouse (Model BRH 1001-203) with a rated capacity of freezing one ton of ice in 24 hours. Approximately 500 feet of copper coil were suspended in the water of the bottom reservoir. The copper coil was coated with several layers of a clear acrylic plastic (Dem-Kote) manufactured by Dayton Electric Manufacturing Company.

Three standard 48 inches fluorescent light fixtures with 40 watt Sylvania Gro-Lux lamps were mounted about one foot high over the surface of the channel to provide light for photosynthesis.

3. Additional Equipment

The spectrophotometer employed in the determination of phosphate and nitrate was a Coleman Model 14. The readings for hydrogen-ion concentration were measured on a Beckman Model 76 Expandable Scale pH Meter and a Beckman Model N-1 pH Meter. The Wollensak Fastax Exposure Meter Weston Model 755 was used to measure light intensity. And all weighings were made on a Ainsworth Type 10 Analytical Balance.

All glassware used was washed in the dichromate acid cleaning solution for two hours, rinsed throughly in tap water and in distilled water. Subsequent washing was the same as above with the exception that acid cleaning was used only periodically.

B. Materials

1. Cladophora

Cladophora fracta used in this study was obtained from Lake Ontario and cultured in the Sanitary Engineering Laboratory at Syracuse University. Cladophora glomerata was obtained from Butternut Creek at the bridge on Jamesville Road 1.6 miles south of Dewitt, New York. The filaments of Cladophora glomerata were removed from rocks and cultured in the "artificial stream" for these experiments.

Butternut Creek is relatively unpolluted for the first 15 miles from its source. Then the stream flows through the residential and industrial area around Syracuse, where it receives raw and treated sewage and industrial waste. The sampling point of Butternut Creek (Figure 5) was Ont. 66-11-P26-37-6(13.9) of the index number of New York Waters Index System (N.Y. State Dept. of Health, 1957). The creek is 30 feet wide, two to three feet deep, swift current, rocky bottom, no sludge deposits, and clear water.

The Village of Jamesville is just above the sampling station. Jamesville has a population of about 1,000. Residential and commercial buildings discharge septic tank effluent or raw sewage to storm sewers or direct to Butternut Creek. The Butternut Creek water is classified as Class C for fishing. (N.Y. State Dept. of Health, 1957).

Figure 5. Sampling station, Cladophora glomerata growing in Butternut Creek, near Jamesville, New York.

2. Medium

The synthetic medium (M-1) used in Phase 1 had the following ingredients dissolved in distilled water: HNO_3, 500 mg/l; $K_2HPO_4 \cdot 3H_2O$, 130 mg/l; $MgSO_4 \cdot 7H_2O$, 100 mg/l; $CaCl_2 \cdot 2H_2O$, 130 mg/l; $FeC_6H_5O_7 \cdot 5H_2O$, 6 mg/l and $MnCl_2 \cdot H_2O$, 2 mg/l. M-2 through M-7 consisted of one less ingredient. Table 4 shows the ingredients and their concentrations in the synthetic media M-1 through M-7. All media were adjusted to pH 7.5 with 10 per cent NaOH solution.

The media used in Phase 2 were different nitrate and phosphate concentration combinations used with a synthetic basal medium. The basal medium consists of the following: $CaCl_2$, 25 mg/l; $MgSO_4 \cdot 7H_2O$, 100 mg/l; $MnCl_2 \cdot 4H_2O$, 2 mg/l; $Fe_2Cl_3 \cdot 6H_2O$, 3 mg/l; and $H_2C_4H_4O_6$, 4 mg/l. Chemicals $NaNO_3$ and $K_2HPO_4 \cdot 3H_2O$ were used as the sources of nitrate and phosphate. The pH of the medium was adjusted to 7.5 as above. All media used in these experiments were not considered free of microorganisms,but were prepared just before each test to minimize the effects of bacteria as well as other contaminations.

C. Procedures

Three phases have been carried out in this study. The procedures for Phases 1 and 2 were essentially the same, except Cladophora fracta and Cladophora glomerata were used in Phases 1 and Phase 2, respectively.

TABLE 4.

COMPOSITION AND CONCENTRATION OF MEDIA M-1 THROUGH M-7

Chemical	Concentration, mg/l	Medium, adjusted to pH of 7.5 with 10% NaOH solution						
		M-1	M-2	M-3	M-4	M-5	M-6	M-7
HNO_3	500	X		X	X	X	X	X
$K_2HPO_4 \cdot 3\ H_2O$	130	X	X		X	X	X	X
$MgSO_4 \cdot 7H_2O$	100	X	X	X		X	X	X
$CaCl_2 \cdot 2H_2O$	130	X	X	X	X		X	X
$FeC_6H_5O_7 \cdot 5H_2O$	6	X	X	X	X	X		X
$M_7Cl_2 \cdot 4H_2O$	2	X	X	X	X	X	X	

Phases 1 and 2. The Respiration and Photosynthetic Rates of Cladophora fracta and Cladophora glomerata under Laboratory Conditions:

Barcroft respirometers (B. manometers, Figure 4) were employed for the purpose of measuring the respiration and photosynthetic rates of Cladophora fracta and Cladophora glomerata under laboratory conditions. The Barcroft respirometer is a closed system formed by two flasks connected by a manometer. One flask contains the algae being studied and is called the reaction vessel. The other flask, free from the algae is called the compensation vessel. It serves to compensate for any changes in temperature and barometric pressure during the course of an experiment.

Three milliliters of a medium were pipetted into both reaction and compensation vessels. Mats of Cladophora were washed several times with distilled water to remove any detritus and blotted with tissue. Filaments of Cladophora were then added to the reaction vessel. An attempt was made to keep the dry weight of the algae between three and six milligrams. A small piece of rubber (about 20 to 40 cubic mm.) with approximately the same volume as that of the algae, was placed into the compensation vessel to make the volumes of liquid in both vessels the same.

The vent tubes of the vessels and the joints of the manometers were lubricated with a light coat of grease. Then the

flasks were mounted and submerged approximately two centimeters below the manometer-flask joint in the constant temperature bath of the Braun's Warburg Apparatus (Figure 3). The constant temperature bath was kept at 17± 0.01°C for all experiments. This temperature was reported to be optimum for the growth of Cladophora (Turano, 1966).

In Phase 1, the metabolic rates of Cladophora fracta were measured both without oscillation and oscillated at 120 strokes per minute. Experiments in Phase 2 were conducted only with oscillation of 120 strokes per minute even though the Warburg apparatus provides six shaking frequencies of 80, 100, 120, 140, 150, and 160 strokes per minute. To select 120 strokes per minute as the experimental shaking speed was based on: 1) the apparatus was used previously at this speed by Dr. Charles E. Smith, Jr.; 2) the algae kept well in suspension at this speed; 3) the algal cell did not break down at this speed.

The Barcroft manometers were the differential type and contained Brodie's manometer fluid (P_o = 10,000 mm.) The manometer fluid was adjusted to a reference point (15.0 cm. mark) and the system was allowed to come to thermal equilibrium. After a few minutes the stop cocks on each manometer were closed simultaneously. The readings of the manometer fluid levels after twenty minutes represent the initial reading for the dark or respiration period. After a certain time (one hour) a second reading was made. Then the lights

were turned on and after twenty minutes the initial readings for the photosynthetic period were taken. After a measured time (one hour), a second photosynthetic reading was recorded and the lights were turned off. Procedures as above were repeated to make both second respiration and photsynthetic measurements. The chart shown in Table 5 indicates these experiments.

After the final photosynthetic reading was recorded, filaments of the Cladophora were removed from the vessel and blotted with paper towels. Then the algae were dried at a temperature of 103 $^{\circ}$ C for one hour, cooled and weighed, ashed at 600 $^{\circ}$ C for 30 minutes, and reweighed to determine the total, organic and inorganic fractions of the Cladophora.

The following equation (Umbreit et al., 1964) was used for calculating oxygen consumption and oxygen production of Cladophora:

$$X = h\left[\left(1 + \frac{AP_{\circ}}{2V_g}\right)\left(\frac{V_g \frac{273}{T} + V_f \alpha}{P_{\circ}} + \frac{A}{2}\frac{273}{T}\right)\right]$$

$$= hk$$

where

X = The amount of oxygen consumed or produced in the reaction vessel, µl

h = Manometer reading in mm. (the difference in height of the manometer liquid in limbs of the manometer).

Notations and theory of the equation, see Appendix.

TABLE 5

METHOD USED FOR DETERMINING RESPIRATION AND PHOTOSYNTHETIC RATES OF CLADOPHORA BY MEANS OF THE DIFFERENTIAL RESPIROMETER

Conditions	Dark	Light Constant intensity	Dark	Light Constant intensity
Processes	Respiration	Photo and Resp.	Respiration	Photo and Resp.
Time	I — T — I — T — I — T — I — T			
	I: 15 - 30 min., 20 min. average T: 30 -120 min., 60 min. average			
Manometer readings taken	↑ R_1 ↑	↑ $R_1 + P_1$ ↑	↑ R_2 ↑	↑ $R_2 + P_2$

Respiration measurement is necessary to obtain the true rate of photosynthesis. The apparent photosynthetic rate, observed during the light period, will be less than the actual one by the rate of respiration. The rate is approximated by taking an average of the rates of respiration observed in the two dark periods. Therefore, the actual rate of photosynthesis is:

$$\text{Actual photosynthetic rate} = \frac{1}{2}(R_1 + R_2 + P_1 + P_2)$$

R_1 and R_2 represent the two dark periods, while P_1 and P_2 represent the two light periods. The respiration and photosynthetic rates are expressed in units of microliters of oxygen per hour per milligram ash-free dry weight of algae (µl of O_2/hr/mg ash-free dry wt.). The oxygen volumes are corrected to standard temperature and pressure, 0 ° C and 760 millimeters of mercury.

Phase 3. Respiration and Photosynthetic Rates of Cladophora glomerata under Natural Conditions:

This series of experiments was intended to determine the respiration and photosynthetic rates of Cladophora glomerata under natural conditions once a week from May 10 through June 14, 1967 in Butternut Creek, Jamesville, New York. The site Ont. 66-11-P26-37-6(13.9) (N.Y. State Dept. of Health, 1957).

The Butternut Creek water was filtered through a type HA millipore filter to remove all particulate matter. The filtered

water was added to each of 16 BOD bottles, eight clear and eight covered with aluminum foil. Mats of Cladophora glomerata were removed from rock on which they were growing and were rinsed several times with filtered stream water to remove any foreign forms. And the mats were blotted with paper towels. Then filaments of the Cladophora were placed in 50 ml. graduated tubes and, when two ml. of water had been displaced, this quantity of algae was added to one of the bottles. All BOD bottles were put into tube baskets which sat on the stream bed at a depth of 30 to 40 cm. for a two-hour period.

The dissolved oxygen content of the water in the bottles was measured by the Alsterberg azide modification of the Winkler method. Measurements were made in each bottle which contained Cladophora glomerata at the end of the two-hour period as well as in four blanks at the beginning and the end of the experiment. Temperatures and light intensities of air and water were recorded several times during each series of experiments.

Each Cladophora sample was removed from the bottles, and was weighed, ashed, reweighed as Phases 1 and 2. The metabolic rates were expressed as µl O_2/hr/mg ash-free dry wt. and were determined by the following equation:

$$\text{RATE} = \frac{(0.3\ \text{l})(\text{DO sample} - \text{DO blank, mg/l})}{(\text{Ash-free dry wt. of algae, mg})(\text{Test period, Hr})} \times \frac{(22{,}400\ \text{ul})}{(32\text{mg})} \times \frac{237.2\ ^{\circ}\text{K}}{(\text{Temp.}\ ^{\circ}\text{K})}$$

The pressure correction is not considered in the above equation.

IV. RESULTS

Phase 1. Respiration and Photosynthetic Rates of Cladophora fracta under Laboratory Conditions.

In this study, experiments were carried out in 19 duplicates for each medium at 17° C under constant illumination of 1,100 f-c and dark conditions with and without oscillation. The respiration and photosynthetic rates of Cladophora fracta in synthetic media by Warburg studies are shown in Tables 6 and 7. Table 6 presents results for flasks shaken at 120 strokes per minute, while Table 7 is for flasks having no oscillation. Figure 6 shows the apparent respiration and photosynthetic rates of Cladophora fracta under laboratory conditions. Each value is the average of 19 determinations.

The data collected in these determinations were evaluated with statistical analysis by means of the analysis of variance. Since two-way classification of the variance (Dixon et al.1957) has been designed for the experiments, ANOVA 2 WAY of the Tape Storage and Retrieval (TSAR) system (Gabor and Carlitz, 1965) on an IBM 7074 computer was employed to analyze the results of the experiments. Table 8 presents the programming of the analysis for Phase 1. The outputs of the analysis are given in Tables 9 and 10.

The mean rates of both respiration and photosynthesis of Cladophora fracta in each medium have been calculated. The

TABLE 6

THE RESPIRATION AND PHOTOSYNTHETIC RATES OF CLADOPHORA FRACTA IN SEVEN SYNTHETIC MEDIA* OSCILLATED AT 120 STROKES PER MINUTE

	M-1		M-2		M-3		M-4		M-5		M-6		M-7	
	RR	PR	RR	PR	RR	PR	RR	PR	RR	PR	RR	PR	RR	PR
	1.38	5.15	.40	1.33	.72	2.24	1.24	4.56	1.04	5.48	1.30	2.33	1.92	3.52
	.67	3.81	.65	1.58	.87	2.15	.70	3.45	1.20	4.67	1.32	2.88	1.23	3.81
	1.80	5.62	.75	1.50	.90	1.89	.93	5.10	1.22	4.87	1.72	3.72	1.83	3.46
	1.48	3.93	.70	1.98	1.02	2.51	.67	4.00	1.37	6.40	1.34	3.07	1.86	3.30
	1.70	4.75	.72	3.45	.98	2.77	.55	5.45	1.72	5.88	1.03	2.50	1.50	4.72
	1.70	5.50	1.52	3.71	1.11	2.02	.51	2.87	1.40	5.22	1.08	3.75	1.26	3.08
	1.63	4.80	1.42	2.85	.98	1.94	.71	2.41	1.18	5.22	1.03	3.86	1.45	3.05
	1.88	3.90	1.34	3.18	1.54	1.99	.65	2.66	1.28	5.33	1.29	3.45	1.36	3.21
	1.02	4.13	1.52	3.13	.98	1.88	1.33	2.46	1.68	6.77	1.08	2.13	1.93	3.96
	2.05	4.44	1.28	2.47	.62	1.36	1.11	2.06	1.08	5.40	.94	2.24	1.26	2.90
	.95	4.28	1.38	4.05	1.24	1.94	.74	2.83	1.28	4.88	1.11	3.45	1.20	4.00
	1.52	3.83	1.25	2.66	.94	1.76	1.26	3.84	.80	4.47	1.41	3.35	1.24	2.70
	.90	2.67	.72	1.44	1.19	1.80	.74	2.14	1.40	6.00	1.42	3.00	1.66	3.05
	1.43	3.65	1.69	3.16	.81	1.46	1.63	2.63	1.05	4.35	1.30	3.16	1.50	3.52
	.99	2.95	.64	2.11	1.05	2.15	.98	1.92	1.69	7.97	.93	3.57	1.85	3.40
	1.66	4.19	1.03	2.18	.67	1.62	.59	2.24	.82	3.82	1.19	4.12	1.08	3.65
	1.93	3.87	.96	2.88	1.04	2.04	.56	2.28	2.58	7.35	1.79	2.77	.99	2.82
	1.42	4.03	1.41	3.03	1.33	2.40	.96	2.48	.85	4.37	1.50	2.30	1.36	4.60
	.85	3.57	1.31	2.71	1.41	2.32	1.33	4.20	1.93	6.78	1.61	3.95	.68	3.24
A	1.42	4.16	1.09	2.60	1.02	2.01	.91	3.13	1.34	5.54	1.28	3.14	1.42	3.44
σ	.41	.76	.38	.79	.24	.35	.32	1.08	.43	1.11	.25	.62	.34	.55

*M-1 through M-7

RR = Respiration rate -- µl 0_2/hr/mg ash-free dry wt.

PR = Photosynthetic rate -- µl 0_2/hr/mg ash-free dry wt.

A = Average

σ = Standard deviation

TABLE 7

THE RESPIRATION AND PHOTOSYNTHETIC RATES OF CLADOPHORA FRACTA IN SEVEN SYNTHETIC MEDIA* WITHOUT OSCILLATION

	M-1		M-2		M-3		M-4		M-5		M-6		M-7	
	RR	PR	RR	PR	RR	PR	RR	PR	RR	PR	RR	PR	RR	PR
	.57	2.45	.68	2.36	.90	2.25	.98	4.30	.83	3.65	.65	2.60	.69	1.63
	.82	2.05	.64	1.98	.71	2.94	1.06	3.17	.63	3.45	.46	1.53	.69	1.70
	.72	2.38	.59	2.60	.96	2.11	.97	2.33	.52	2.10	.57	1.27	1.10	2.42
	1.86	3.35	.69	1.60	.88	1.76	.80	4.00	.63	2.27	.74	1.40	.63	2.23
	.62	3.32	.52	2.38	.84	1.26	1.34	2.95	.74	2.22	.93	3.76	.48	2.46
	1.68	3.82	.50	1.80	.64	1.28	.78	2.40	1.36	2.01	.72	2.66	.94	2.57
	1.69	3.44	.93	2.01	.46	1.15	.97	2.22	2.20	4.00	.74	1.57	.61	1.96
	.59	3.22	.97	2.38	.83	1.54	.61	2.27	1.64	2.96	1.35	2.67	1.57	2.60
	1.14	1.53	1.04	1.85	.68	1.46	.74	3.11	1.73	4.16	.47	2.00	.79	1.94
	.72	1.39	.90	2.40	.73	1.46	.93	2.64	1.56	5.11	1.06	1.66	.68	2.52
	.77	1.40	1.00	1.75	.38	.95	1.12	2.87	1.84	4.45	.71	2.64	1.30	2.64
	.85	2.06	.63	2.18	.77	1.53	1.08	2.22	2.11	3.66	1.06	2.18	.65	1.88
	.60	2.38	.76	1.42	.64	1.08	1.28	3.00	1.92	3.21	.90	2.02	1.24	2.76
	1.46	2.50	.72	1.44	.55	1.18	1.08	2.38	1.04	3.73	.81	2.43	1.00	2.14
	1.12	1.95	.80	1.93	.90	2.05	.91	2.84	.68	3.21	.93	4.15	.86	1.75
	1.98	2.90	1.06	2.60	.70	1.28	1.10	2.53	1.61	5.03	.87	3.29	.95	2.35
	1.86	4.55	.88	2.02	.66	1.57	.79	3.26	1.53	4.83	.92	1.96	.59	1.66
	1.78	2.60	.64	1.80	.80	1.41	1.23	2.92	1.36	3.14	.91	2.34	.89	2.52
	1.31	2.18	.98	1.81	.66	1.12	1.01	2.73	.94	3.63	1.31	2.68	.82	2.14
A	1.17	3.38	.79	2.31	.72	1.78	.99	2.99	1.31	4.53	.85	2.75	.87	2.84
σ	.51	1.12	.18	.68	.15	.48	.19	.86	.54	.44	.24	.80	.28	.79

* = M-1 through M-7

PR = Photosynthetic rate, μl O_2/hr/mg ash free dry wt.

RR = Respiration rate, μl O_2/hr/mg ash free dry wt.

A = Average. σ = Standard Deviation

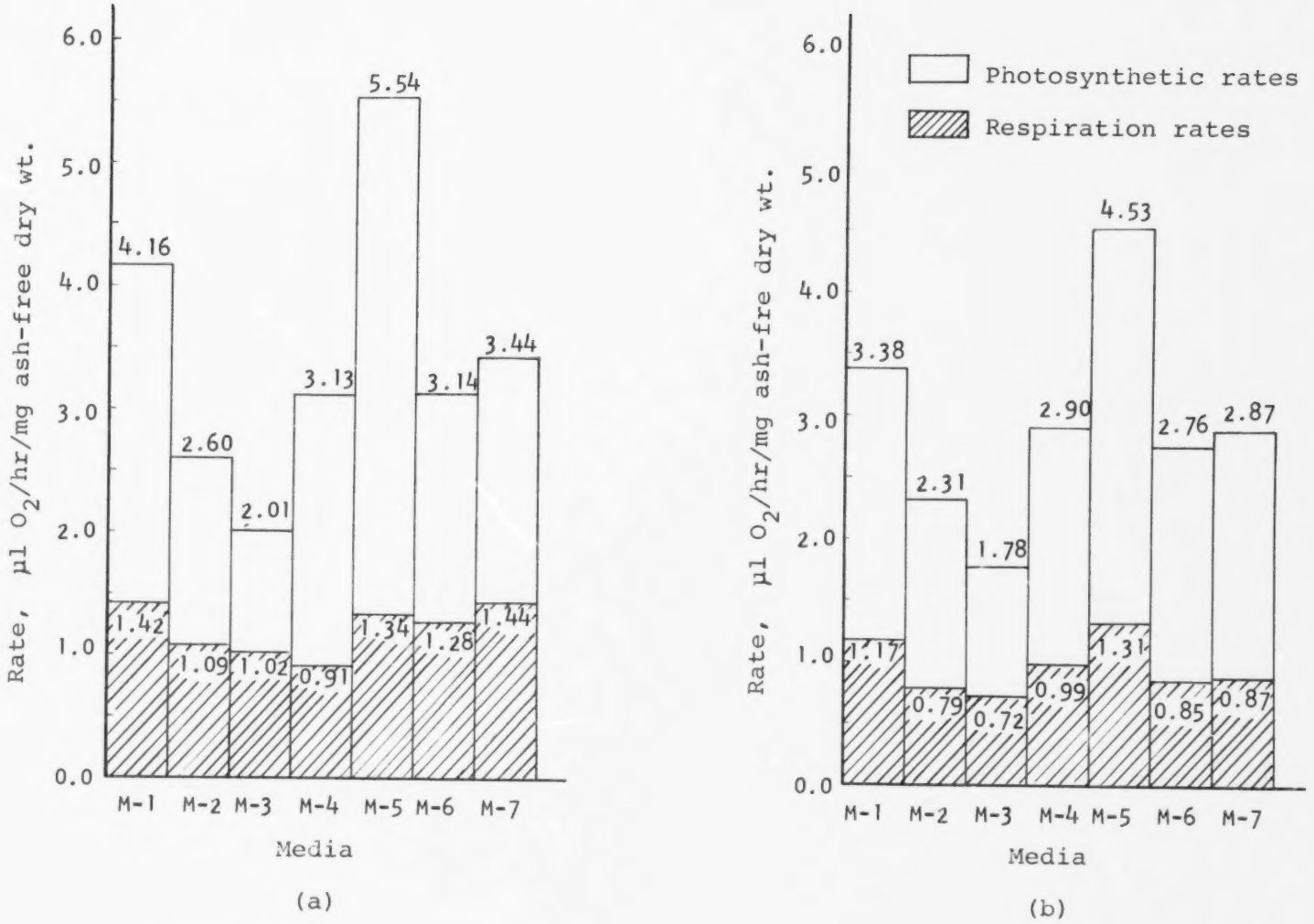

Figure 6. Respiration and photosynthetic rates of Cladophora fracta in seven synthetic media under laboratory conditions.

a) Oscillation at 120 strokes per minute.

b) No oscillation.

Each value is the average of 19 measurements.

TABLE 8

PROGRAMMING FOR PHASE 1

```
***  LIII,  SHUNNDAR RR+PR OF C. F. IN M1-M71-M7      10TCIVLE      561
*    EXECUTE  (TSAR)
     1
IVAN 1      RUN
ORIGINAL
ANOVA 2WAY RR + PR OF C. F. IN M1-M7 WI AND WO VIB
OBS 1-3 VIB 6 MED 11 RR 16-19 PR 21-24

{1    V    1    1.38  5.15\
{                          } 266 data cards
END
DATA        RUN
ANOVA 2WAY RR+PR OF C. F. IN M1-M7 WI AND WO VIB SHUNNDAR LIN APR. 12 67
OBS VIB MED RR PR

GROUP 1= 0 WITH MED EQUAL 1.
GROUP 2= 0 WITH MED EQUAL 2.
GROUP 3= 0 WITH MED EQUAL 3.
GROUP 4= 0 WITH MED EQUAL 4.
GROUP 5= 0 WITH MED EQUAL 5.
GROUP 6= 0 WITH MED EQUAL 6.
GROUP 7= 0 WITH MED EQUAL 7.
GROUP11= 0 WITH VIB EQUAL  'V
GROUP12= 0 NOT 11
0     ANOVA 2WAY RR 1 2 3 4 5 6 7
      11 12
0     ANOVA 2WAY PR 1 2 3 4 5 6 7
      11 12
```

TABLE 9. TSAR OUTPUT 1 FOR PHASE 1

TAPE STORAGE AND RETRIEVAL SYSTEM — SYRACUSE UNIVERSITY SYSTEMS

ANOVA 2WAY RR+PR OF C. F. IN M1-M7 WI AND WO VIB — SHUNNDAR LIN APR.12 67

GROUP 0, N = 266

TWO WAY ANALYSIS OF VARIANCE FOR VARIABLE RR

GROUP	1	2	3	4	5	6	7	TOTAL
11 N	19	19	19	19	19	19	19	133
AVE	1.4189	1.0889	1.0216	0.9063	1.3458	1.2837	1.4295	1.2135
SD	0.4090	0.3824	0.2443	0.3293	0.4337	0.2509	0.3456	0.3916
12 N	19	19	19	19	19	19	19	133
AVE	1.1653	0.7858	0.7205	0.9884	1.3089	0.8479	0.8674	0.9549
SD	0.5127	0.1800	0.1523	0.1904	0.5420	0.2417	0.2796	0.3826
TOT	38	38	38	38	38	38	38	266
AVE	1.2921	0.9374	0.8711	0.9474	1.3274	1.0658	1.1484	1.0842
SD	0.4751	0.3324	0.2522	0.2686	0.4845	0.3283	0.4210	0.4075

SOURCE	DF	SS	MS	F	P
COLUMNS	6	7.3163	1.2194	9.7558	
ROWS	1	4.4488	4.4488	35.5924	
ROWSRROR	6	2.7794	0.4632		
ERROR	252	29.4688	0.1169	0.3420 = SQRT WITHIN	

TABLE 10. TSAR OUTPUT 2 FOR PHASE 2

TAPE STORAGE AND RETRIEVAL SYSTEM — SYRACUSE UNIVERSITY SYSTEMS

ANOVA 2WAY RR+PR OF C. F. IN M1-M7 WI AND WO VIB — SHUNNDAR LIN APR.12 67

GROUP 0. N = 266

TWO WAY ANALYSIS OF VARIANCE FOR VARIABLE PR

GROUP	1	2	3	4	5	6	7	TOTAL
11 N	19	19	19	19	19	19	19	133
AVE	4.1616	2.6000	2.0126	3.1358	5.5384	3.1368	3.4732	3.4369
SD	0.7639	0.7943	0.3491	1.0785	1.1081	0.6278	0.5534	1.3168
12 N	19	19	19	19	19	19	19	133
AVE	2.6037	2.0163	1.5463	2.8495	3.5168	2.3584	2.2037	2.4421
SD	0.8473	0.3608	0.4921	0.5670	0.9524	0.7749	0.3691	0.8725
TOT	38	38	38	38	38	38	38	266
AVE	3.3826	2.3082	1.7795	2.9926	4.5276	2.7476	2.8384	2.9395
SD	1.1209	0.6765	0.4826	0.8622	1.4449	0.7997	0.7931	1.2212

SOURCE	DF	SS	MS	F
COLUMNS	6	171.4803	28.5801	46.6923
ROWS	1	65.8120	65.8120	107.5183
ROWSRROR	6	23.2161	3.8694	
ERROR	252	134.6982	0.5345	

0.7311 = SQRT WITHIN

problem then is whether all means are equal or not. This was evaluted by the F-distribution. Assuming the means of all catagories are equal, compute F, then accept or reject the hypothesis by comparing with the critical region within the level of significance.

From the analysis for the respiration rates of Cladophora fracta, to test for column effects, we obtained (Table 9),

$$F = 9.76$$

and the critical regions (Dixon and Massey, 1957)

$$F_{.95}(6,258) = F_{.95}(6,\infty) = 2.10$$

$$F_{.99}(6,258) = F_{.99}(6,\infty) = 2.80$$

This value is significant, and we reject the hypothesis of equal column means. To test for row effects, we had (Table 9),

$$F = 35.59$$

and had (Dixon and Massey, 1957)

$$F_{.95}(1,258) = 3.84$$

$$F_{.99}(1.258) = 6.63$$

This is also significant at both the five and one per cent probability levels.

To test for column and row effects for the photosynthetic rates of the algae, we had (Table 10),

$$F_c = 46.69 > F_{.99}(6,258) > F_{.95}(6,258)$$

and $$F_r = 107.52 > F_{.99}(1,258) > F_{.95}(1,258)$$

The above indicates that we may reject the hypothesis of equal column and row means. In other words, there are significant differences among column categories and row categories with 99 per cent confidence.

In the media M-1 through M-7 (Table 6) oscillated at 120 strokes per minute, the respiration rates of Cladophora fracta were 1.42, 1.09, 1.02, 0.91, 1.34, 1.28, and 1.42 µl O_2/hr/mg ash-free dry wt., respectively; while the photosynthetic rates 4.16, 2.60, 2.01, 3.13, 5.54, 3.14, and 3.44 µl O_2/hr/mg ash-free dry wt., respectively. The flasks which contained the same media as above and were not oscillated had algal respiration rates as follows: 1.17, 0.79, 0.72, 0.99, 1.31, 0.85, and 0.87 µl O_2/hr/mg ash-free dry wt.,respectively; and 3.38, 2.31, 1.78, 2.99, 4.53, 2.75, and 2.84 µl O_2/hr/mg ash-free dry wt. of photosynthetic rates respectively for the media M-1 through M-7.

The highest metabolic rates (Figure 6) were found in medium M-5 in which there was no $CaCl_2$ for both conditions - with and without oscillation. The lowest values were observed in both media M-2 and M-3 which were free of nitrate and phosphate, respectively; the photosynthetic rates in these two media were only about one-half and one-third of that in the medium M-5.

Phase 2: Respiration and Photosynthetic Rates of Cladophora glomerata under Laboratory Conditions.

In the course of this phase, experiments were also conducted with the Warburg apparatus using manometric techniques.

The temperature was kept constant at 17° C. The vessels containing Cladophora glomerata were exposed at about 1,400 f-c light intensity as well as under dark conditions. All tests were carried out with oscillation at 120 strokes per minute and seven duplicates for each medium which were prepared freshly for different combinations of phosphate and nitrate.

The observed data are presented in Table 11. In each cell of Table 11, the first and second columns give the respiration and photosynthetic rates of Cladophora glomerata in the indicated nitrate-phosphate concentration with the basal medium, respectively. Tables 12 and 13 show respectively the average rates of respiration and photosynthesis from the observed data in Table 11. Each value is the average of seven measurements. These average rates are demonstrated graphically in Figures 7 and 8. Figure 7 (Figure 7-a through Figure 7-l) present the metabolic rates of Cladophora glomerata versus phosphate concentration for a given concentration of nitrate. Figure 8 presents the rates versus nitrate concentration without phosphate in the media.

The respiration and photosynthetic rates of Cladophora glomerata in medium containing no phosphate and no nitrate were -0.45 and 1.49 µl O_2/hr/mg ash-free dry wt., respectively. This photosynthetic rate was the lowest one in all media used. This respiration rate was also lower than that in other media used except in some media containing no phosphate. It was found that there was a tendency to increase the metabolic rates when the combinations of nitrate and phosphate concentration increased to a certain level,

TABLE 11

THE RESPIRATION AND PHOTOSYNTHETIC RATES OF CLADOPHORA GLOMERATA UNDER VARIOUS CONCENTRATIONS OF NITRATE AND PHOSPHATE WITH THE BASAL MEDIUM.

NO_3* \ PO_4* / Rate	0.00		0.01		0.10		0.50		1.00		2.00		5.00	
	R	P	R	P	R	P	R	P	R	P	R	P	R	P
0.00	.37	1.21	.39	1.58	.45	2.04	.78	1.92	.79	2.70	.65	2.51	.57	1.93
	.56	1.63	.55	1.52	.72	2.46	.83	1.95	.45	1.60	.52	2.30	.62	2.00
	.55	1.52	.72	2.13	.79	3.03	.95	2.88	.93	2.81	.67	2.64	.60	2.46
	.32	1.50	.74	2.15	.73	2.10	.97	2.62	.74	2.22	.82	2.75	.87	2.87
	.56	1.47	.80	2.12	.69	2.22	.88	1.85	1.12	2.77	.65	2.80	.74	3.33
	.31	1.34	.54	1.87	.55	1.70	.61	1.98	.52	2.40	.70	2.44	.63	2.69
	.45	1.76	.32	1.34	.44	1.25	.67	1.88	.65	2.02	.74	2.48	.53	2.02
0.01	.31	1.95	.75	2.68	.71	2.50	.72	2.08	.90	2.75	.65	2.51	.57	2.60
	.35	1.85	.44	1.84	.54	2.11	1.06	3.00	.92	3.10	.52	2.30	.70	2.70
	.23	1.95	.59	1.88	.67	2.43	.68	2.25	.81	2.62	.67	3.07	.50	2.48
	.28	1.78	.89	3.33	.69	2.96	.79	2.61	.77	1.99	.82	2.95	.48	2.52
	.34	2.05	.68	2.42	.49	1.99	.90	2.66	.83	2.50	.65	2.80	.43	2.60
	.33	1.82	.40	1.95	.88	3.11	.89	2.82	1.01	3.17	.70	2.86	.43	2.66
	.22	1.99	.52	2.55	.76	2.02	.70	2.06	.69	2.93	.49	2.02	.60	2.90
0.10	.40	2.02	.51	2.18	.56	2.10	.82	2.67	.66	3.55	.78	2.21	.57	2.76
	.26	1.62	.57	2.00	.75	2.48	.98	2.50	1.20	2.44	.78	3.50	.58	2.47
	.40	2.69	.60	2.96	.61	2.88	1.17	2.84	.68	2.96	.46	3.20	.55	2.57
	.36	2.21	.88	2.57	.68	2.57	.75	2.02	.80	3.62	.89	2.40	.58	2.35
	.40	1.66	.50	2.49	.74	2.40	.96	3.35	.92	2.92	.43	3.45	.48	2.56
	.33	1.34	.52	2.25	.88	2.88	.88	2.75	.64	3.15	.65	3.00	.50	2.56
	.42	2.18	.67	2.55	1.02	2.92	.62	2.66	1.09	2.82	.38	3.02	.58	2.23
0.20	.40	1.80	.40	1.69	.67	2.07	1.06	4.30	.96	2.95	.54	2.06	.77	2.75
	.32	1.76	.43	1.86	.45	2.04	.63	2.72	1.01	3.25	.80	2.81	.57	2.00
	.53	2.06	.66	2.70	.65	1.97	1.04	3.55	.95	2.93	.91	2.74	.66	2.46
	.22	2.03	.55	2.34	.79	2.12	.50	3.75	.80	2.51	.55	2.05	.55	2.44
	.48	2.32	.52	1.85	.73	2.24	.47	3.11	.92	2.97	.72	2.65	.70	3.03
	.45	2.26	.83	2.49	.71	1.90	.86	2.56	1.22	3.57	.44	2.20	.50	2.55
	.32	2.22	.60	2.02	.62	2.90	.73	2.98	.88	3.11	.80	2.88	.55	2.77

R= Respiration rate, µl O_2/hr/mg ash-free dry wt.

P= Photosynthetic rate, µl O_2/hr/mg ash-free dry wt.

*= Unit expressed in mg/l

TABLE 11

THE RESPIRATION AND PHOTOSYNTHETIC RATES OF CLADOPHORA GLOMERATA UNDER VARIOUS CONCENTRATIONS OF NITRATE AND PHOSPHATE WITH THE BASAL MEDIUM (Continued).

NO_3* \ PO_4* / Rate	0.00		0.01		0.10		0.50		1.00		2.00		5.00	
	R	P	R	P	R	P	R	P	R	P	R	P	R	P
0.50	.49	2.28	.36	1.61	.54	2.06	.89	2.66	.86	2.32	.78	2.34	.49	2.25
	.35	1.91	.45	1.66	.44	1.99	.58	3.13	1.11	3.13	.81	2.32	.84	2.32
	.37	2.51	.44	1.68	.77	2.22	.86	3.25	1.22	3.92	.76	2.13	.59	2.52
	.59	2.30	.38	2.25	.48	2.14	.66	2.90	.98	2.88	.63	2.50	.47	2.25
	.24	1.45	.66	2.44	.79	3.55	.68	4.25	1.08	3.34	.74	2.75	.63	2.28
	.59	2.10	.53	2.46	.63	2.05	.50	2.65	.89	2.66	.57	2.28	.64	2.50
	.44	2.20	.99	3.22	.52	2.28	.93	3.33	.98	2.77	.54	2.48	.72	2.82
1.00	.53	2.80	.68	2.78	.64	2.42	.74	2.60	1.08	3.30	1.00	3.15	.55	1.98
	.45	1.68	.44	1.88	.60	2.19	.69	2.72	1.26	3.00	.48	2.20	.59	2.50
	.36	1.92	.48	2.58	.63	2.54	.78	2.76	.98	2.58	.68	3.00	1.00	3.03
	.47	2.33	.40	1.90	.92	2.56	.65	2.78	1.13	2.60	.74	3.08	.83	2.88
	.53	2.11	.48	1.60	.72	2.31	.69	3.23	1.17	3.68	.54	2.46	.66	2.78
	.39	1.74	.54	2.53	.76	2.11	.77	2.25	.92	3.12	.79	2.55	.58	2.45
	.52	2.44	.68	2.22	.69	2.61	.86	2.93	1.10	3.11	.50	2.02	.77	2.90
2.00	.50	2.08	.44	1.41	.51	2.16	.56	2.53	.82	2.93	.59	2.43	.96	3.55
	.66	2.14	.81	2.40	.56	2.07	.92	2.30	1.16	2.70	.66	2.25	1.16	2.88
	.45	1.78	.52	2.18	.73	2.11	.87	2.66	.80	2.64	.55	2.75	1.02	3.62
	.50	2.00	.49	2.26	.66	2.12	.94	3.26	.65	2.40	.75	2.62	.88	3.25
	.65	2.48	.77	2.15	.96	2.51	.71	2.97	.57	2.24	.76	3.23	.76	4.24
	.52	1.88	.33	1.75	.85	3.02	.95	2.82	.70	2.63	.59	2.47	1.17	3.03
	.92	2.42	.80	3.35	.89	2.53	.85	2.83	.83	2.94	.93	3.86	.95	3.02
5.00	.65	2.20	.66	2.20	.75	2.57	.55	2.55	1.02	2.86	.83	3.60	1.25	5.20
	.48	1.55	.72	2.00	.78	1.90	.80	1.98	1.40	3.39	.68	2.22	1.12	4.31
	.53	1.85	.82	2.20	.83	2.26	.70	2.35	.70	3.01	.54	2.62	1.20	3.84
	.59	2.04	.56	2.18	.78	2.25	.56	2.55	1.00	3.43	.67	2.88	.81	4.54
	.88	2.55	.94	2.42	.65	2.82	.48	2.66	.99	3.23	.66	3.46	.97	4.85
	.45	2.00	.39	1.94	.69	2.35	.70	2.38	1.02	2.95	.73	3.18	.89	3.88
	.69	2.70	.57	2.54	.67	2.60	.88	2.77	.97	3.02	.77	3.33	1.51	5.36

R= Respiration rate, µl O_2/hr/mg ash-free dry wt.

P= Photosynthetic rate, µl O_2/hr/mg ash-free dry wt.

*= Unit expressed in mg/l

TABLE 11

THE RESPIRATION AND PHOTOSYNTHETIC RATES OF CLADOPHORA GLOMERATA UNDER VARIOUS CONCENTRATIONS OF NITRATE AND PHOSPHATE WITH THE BASAL MEDIUM (Concluded).

NO_3* \ PO_4* Rate	0.00		0.01		0.10		0.50		1.00		2.00		5.00	
	R	P	R	P	R	P	R	P	R	P	R	P	R	P
10.00	.70	2.80	.38	1.95	.82	2.40	.62	2.81	.90	3.04	.89	3.55	1.48	4.50
	.55	2.14	.50	2.42	.81	2.68	.63	2.93	1.36	3.97	.91	3.85	1.33	4.39
	.71	2.36	.86	2.70	1.01	3.18	.80	2.86	.87	3.74	1.04	4.20	1.02	4.07
	.54	2.44	.72	2.88	.93	3.00	.84	2.43	.87	2.93	1.04	3.52	1.28	3.83
	.32	1.87	.88	3.33	.71	3.23	.62	2.20	1.05	3.14	1.18	4.03	1.01	3.85
	.43	1.93	.77	2.60	.69	2.40	.49	2.44	.88	2.64	1.02	3.75	1.12	3.76
	.55	2.00	.72	2.60	.95	3.00	.85	2.55	.94	3.49	.69	3.63	1.35	3.70
20.00	.84	2.53	.21	2.22	.97	2.87	.98	2.44	1.13	3.73	1.14	4.16	1.29	5.37
	.71	2.42	.48	2.66	.77	2.72	1.01	2.89	1.09	3.45	.99	3.72	1.20	4.45
	.27	1.46	.86	2.85	.82	2.80	1.02	2.83	.72	2.64	.63	3.42	1.09	4.81
	.34	2.05	.84	2.22	1.02	3.22	1.15	2.88	1.00	3.58	.94	3.89	1.06	4.81
	.31	1.85	.48	2.38	.88	2.89	.77	1.93	1.10	3.68	.89	3.95	1.25	5.39
	.60	2.30	.64	2.16	.72	2.46	1.05	3.12	.80	2.48	.84	2.96	1.08	4.55
	.55	2.44	.75	2.68	.65	2.02	.95	3.00	.96	3.58	.97	3.64	1.15	4.89
50.00	.41	2.69	.54	2.68	.77	2.78	.84	2.60	.62	3.11	.82	3.18	1.21	5.02
	.41	1.98	.51	2.42	.53	2.28	.88	2.63	.95	3.85	1.12	3.60	.89	3.66
	.25	1.98	.47	2.66	.64	2.32	.99	2.88	.74	3.41	.95	3.67	.95	4.25
	.26	1.89	.73	2.40	.68	2.83	1.15	2.48	.63	3.76	.92	2.93	.89	3.82
	.39	2.05	.90	2.76	.77	3.33	.85	3.30	1.28	4.60	.88	3.11	.95	4.04
	.27	1.95	.80	2.62	.56	2.77	.87	2.48	.99	3.55	1.18	4.04	1.04	4.12
	.74	2.39	.83	2.36	.71	2.73	1.26	3.52	.84	3.08	.90	3.66	.82	3.03
100.00	.56	2.28	.52	2.09	.52	2.85	.77	2.56	1.13	3.88	.96	3.64	.87	3.35
	.54	2.28	.66	2.42	.47	2.02	.85	2.68	.96	3.66	1.16	3.88	.56	2.82
	.42	2.34	.87	2.50	.78	3.15	.82	3.05	.66	2.96	1.17	3.79	.75	2.99
	.49	1.92	.83	2.98	.69	2.99	1.04	3.20	.55	3.45	.71	3.64	1.06	4.23
	.49	2.42	.66	2.80	.55	2.40	1.02	2.46	1.22	4.99	.86	3.90	.71	2.66
	.33	1.97	.59	2.44	.60	2.80	.89	2.72	.56	3.29	.96	3.42	.93	3.92
	.39	2.42	.50	2.03	.65	2.88	.84	3.05	.69	3.40	.84	3.85	1.11	3.66

R= Respiration rate, µl O_2/hr/mg ash-free dry wt.

P= Photosynthetic rate, µl O_2/hr/mg ash-free dry wt.

*= Unit expressed in mg/l

TABLE 12

AVERAGE RESPIRATION RATES* OF CLADOPHORA GLOMERATA IN DIFFERENT CONCENTRATIONS OF NITRATE AND PHOSPHATE WITH THE BASAL MEDIUM.

NO_3, mg/l \ PO_4, mg/l	0.00	0.01	0.10	0.50	1.00	2.00	5.00
0.00	0.45	0.58	0.62	0.81	0.74	0.68	0.65
0.01	0.29	0.61	0.68	0.82	0.84	0.64	0.53
0.10	0.36	0.61	0.75	0.88	0.86	0.62	0.54
0.20	0.39	0.57	0.66	0.76	0.96	0.68	0.61
0.50	0.44	0.54	0.60	0.73	1.02	0.69	0.63
1.00	0.46	0.53	0.71	0.74	1.09	0.68	0.71
2.00	0.60	0.59	0.74	0.83	0.79	0.69	0.99
5.00	0.61	0.67	0.74	0.67	1.01	0.70	1.11
10.00	0.54	0.69	0.85	0.69	0.98	0.97	1.23
20.00	0.52	0.61	0.83	0.99	0.97	0.91	1.16
50.00	0.39	0.68	0.67	0.98	0.86	0.97	0.96
100.00	0.46	0.66	0.61	0.89	0.82	0.95	0.85

*Expressed in µl O_2/hr/mg ash-free dry wt.

TABLE 13

AVERAGE PHOTOSYNTHETIC RATES* OF CLADOPHORA GLOMERATA IN DIFFERENT CONCENTRATIONS OF NITRATE AND PHOSPHATE WITH THE BASAL MEDIUM.

NO_3, mg/l \ PO_4, mg/l	0.00	0.01	0.10	0.50	1.00	2.00	5.00
0.00	1.49	1.82	2.11	2.15	2.36	2.56	2.47
0.01	1.91	2.38	2.45	2.50	2.72	2.64	2.64
0.10	1.96	2.43	2.60	2.68	3.07	2.97	2.50
0.20	2.06	2.14	2.18	3.28	3.04	2.48	2.57
0.50	2.11	2.19	2.33	3.17	3.00	2.40	2.42
1.00	2.15	2.21	2.39	2.75	3.06	2.64	2.65
2.00	2.11	2.21	2.36	2.76	2.64	2.80	3.37
5.00	2.13	2.21	2.39	2.46	3.13	3.04	4.57
10.00	2.22	2.64	2.84	2.60	3.27	3.79	4.01
20.00	2.15	2.45	2.71	2.72	3.31	3.68	4.90
50.00	2.13	2.56	2.72	2.84	3.62	3.46	3.99
100.00	2.23	2.47	2.73	2.82	3.66	3.73	3.38

*Expressed in µl O_2/hr/mg ash-free dry wt.

Figure 7. Respiration and photosynthetic rates of Cladophora glomerata in different combinations of nitrate and phosphate concentrations under laboratory conditions; (a) through (l) the nitrate concentration varies from 0.00, 0.01, 0.10, 0.20, 0.50, 1.00, 2.00, 5.00, 10.00, 20.00, 50.00, to 100.00 mg/l. Each value is the average of seven measurements.

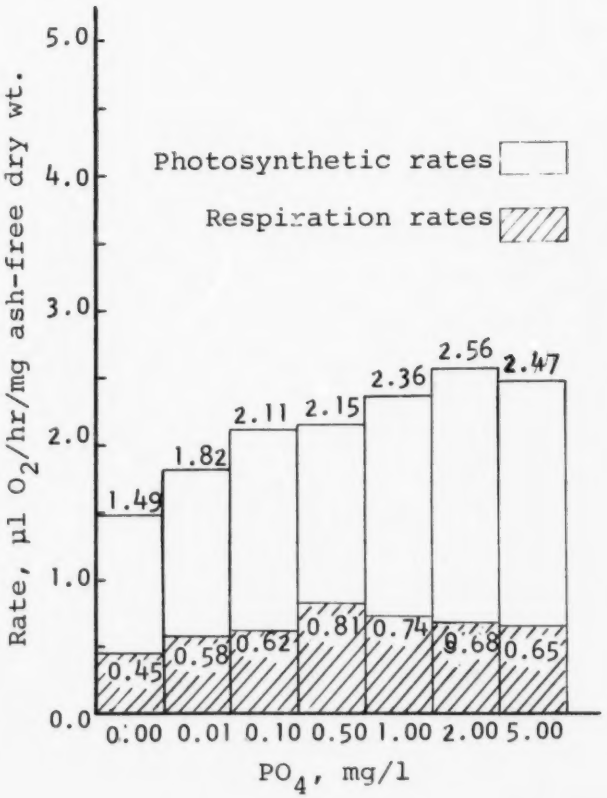

Figure 7-a. NO_3=0.00mg/l

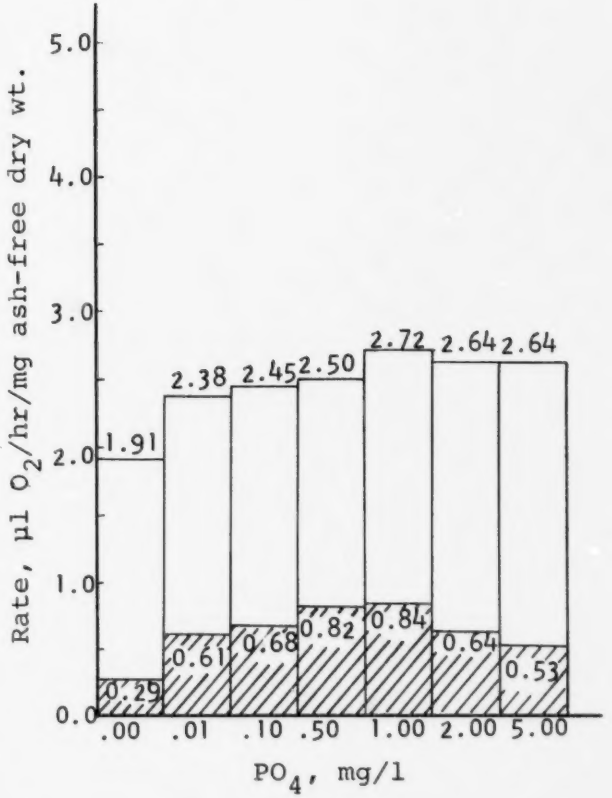

Figure 7-b. NO_3=0.01 mg/l

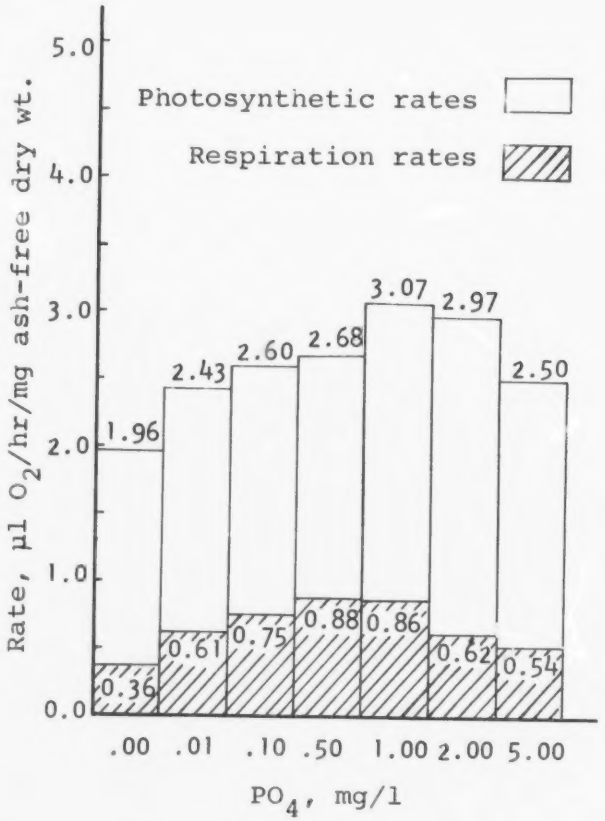

Figure 7-c. NO_3=0.10 mg/l

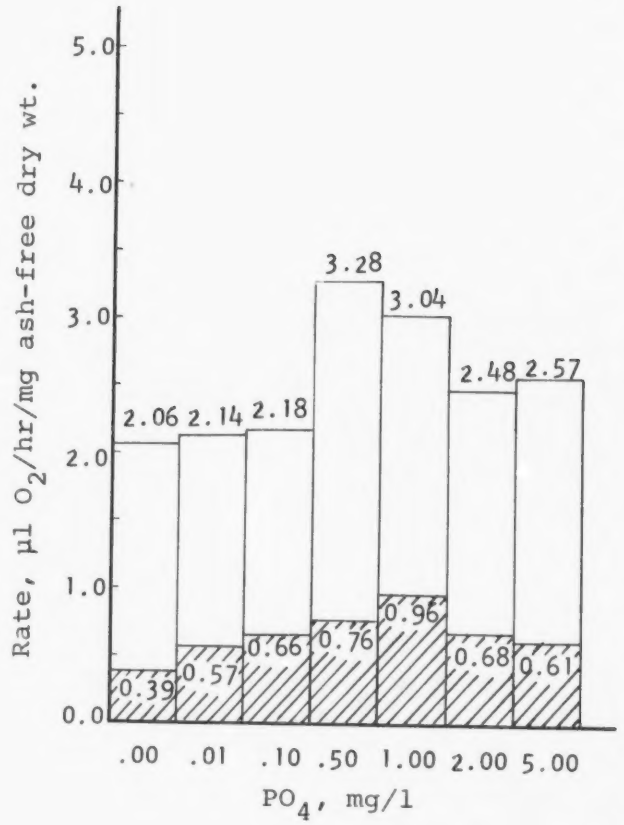

Figure 7-d. NO_3=0.20 mg/l

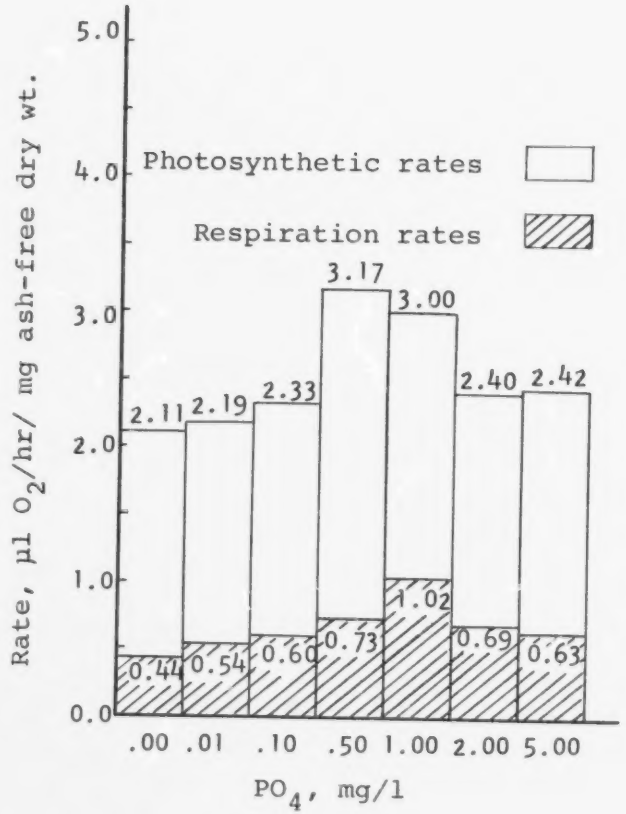

Figure 7-e. NO_3=0.50 mg/l

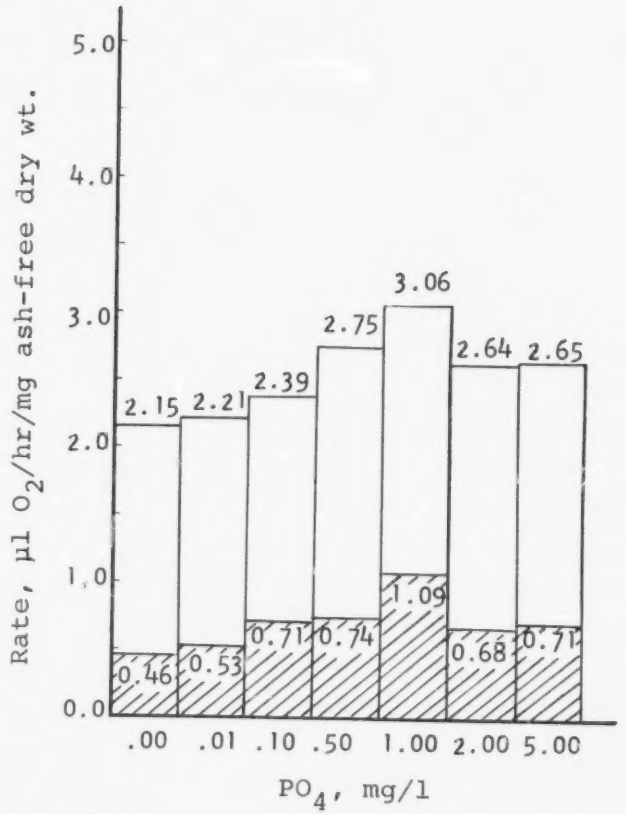

Figure 7-f. NO_3=1.00 mg/l

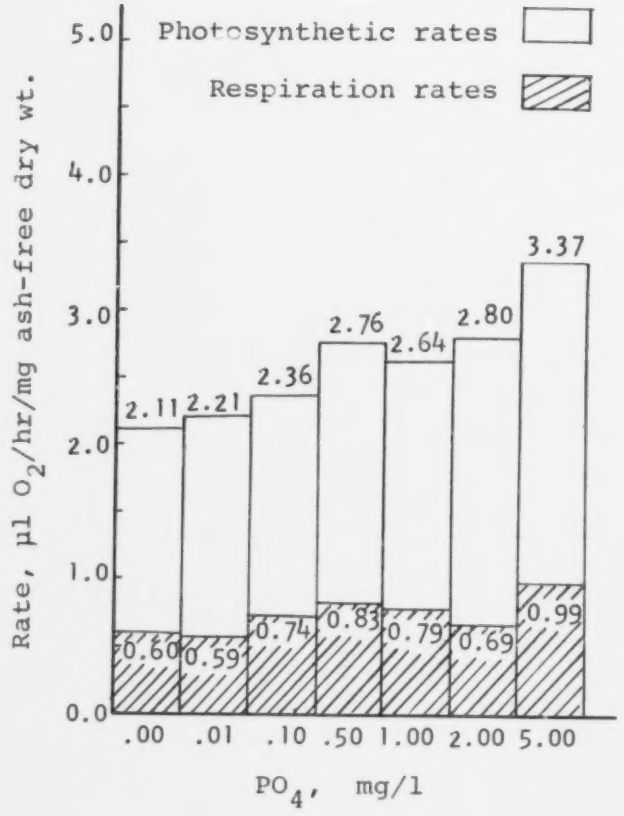

Figure 7-g. NO_3=2.00 mg/l

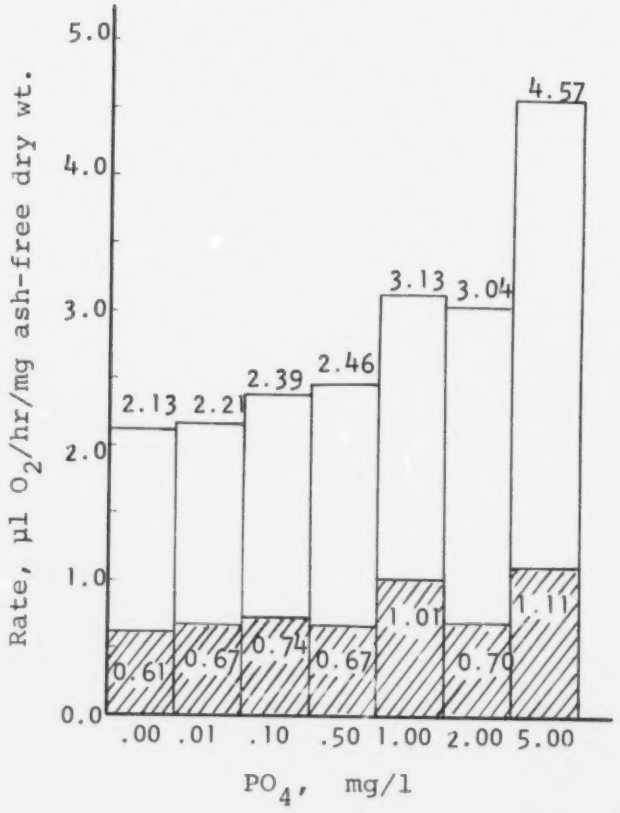

Figure 7-h. NO_3=5.00 mg/l

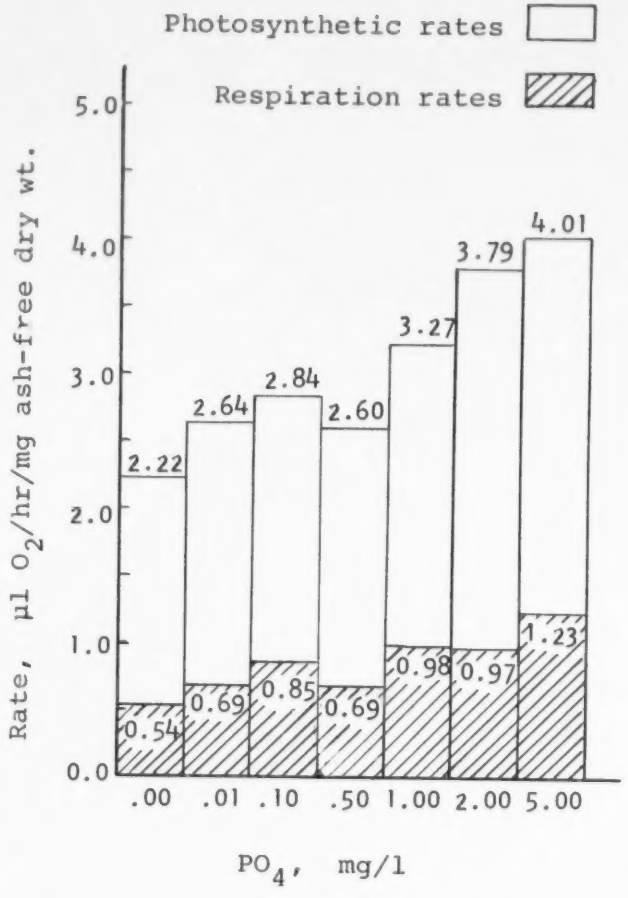

Figure 7-i. NO_3=10.00 mg/l

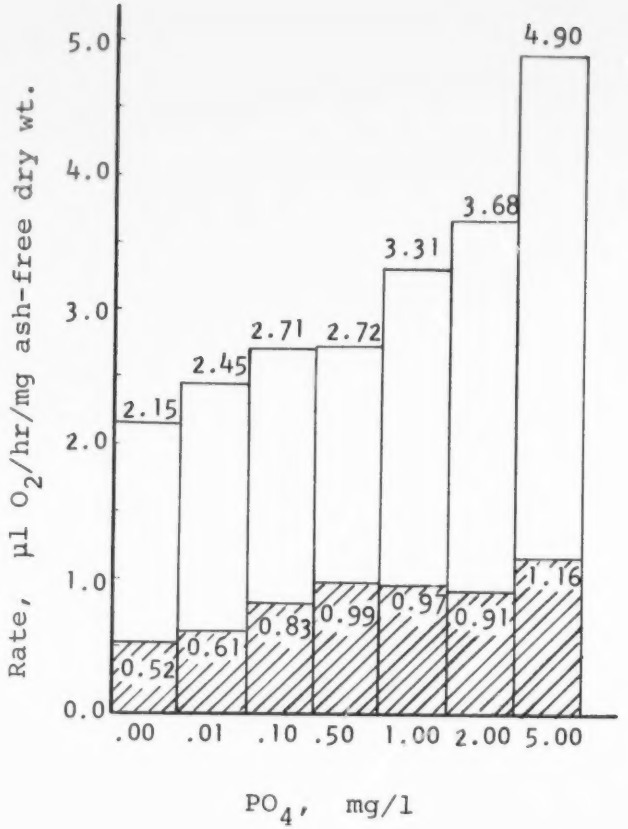

Figure 7-j. NO_3=20.00 mg/l

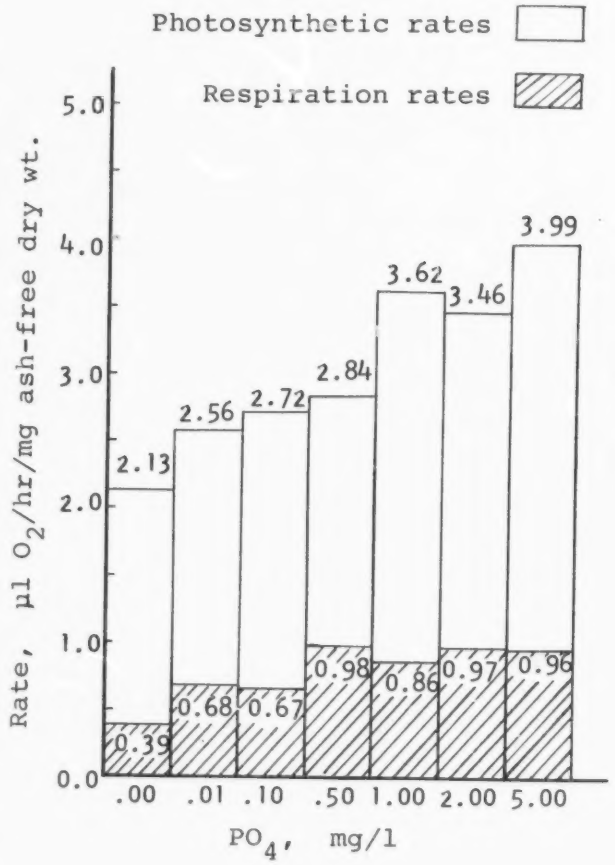

Figure 7-k. NO_3=50.00 mg/l

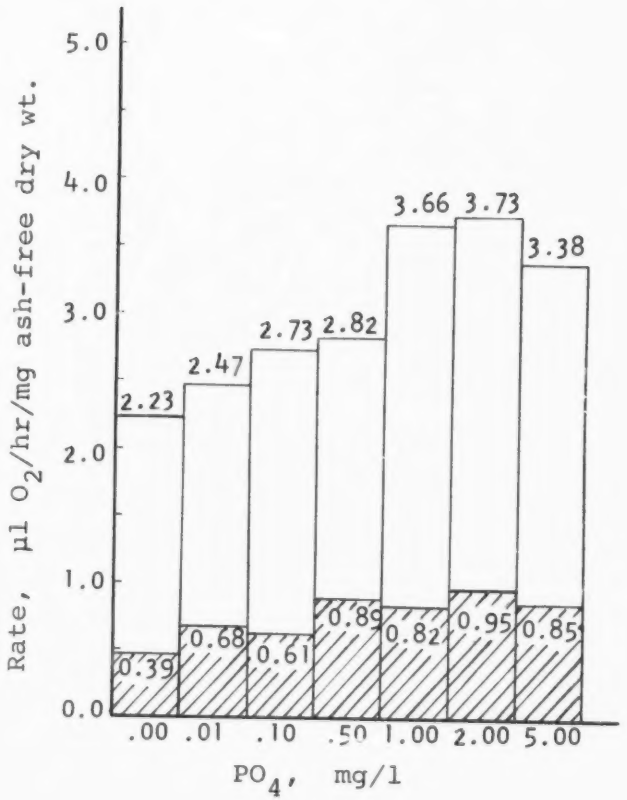

Figure 7-l. NO_3=100.00 mg/l

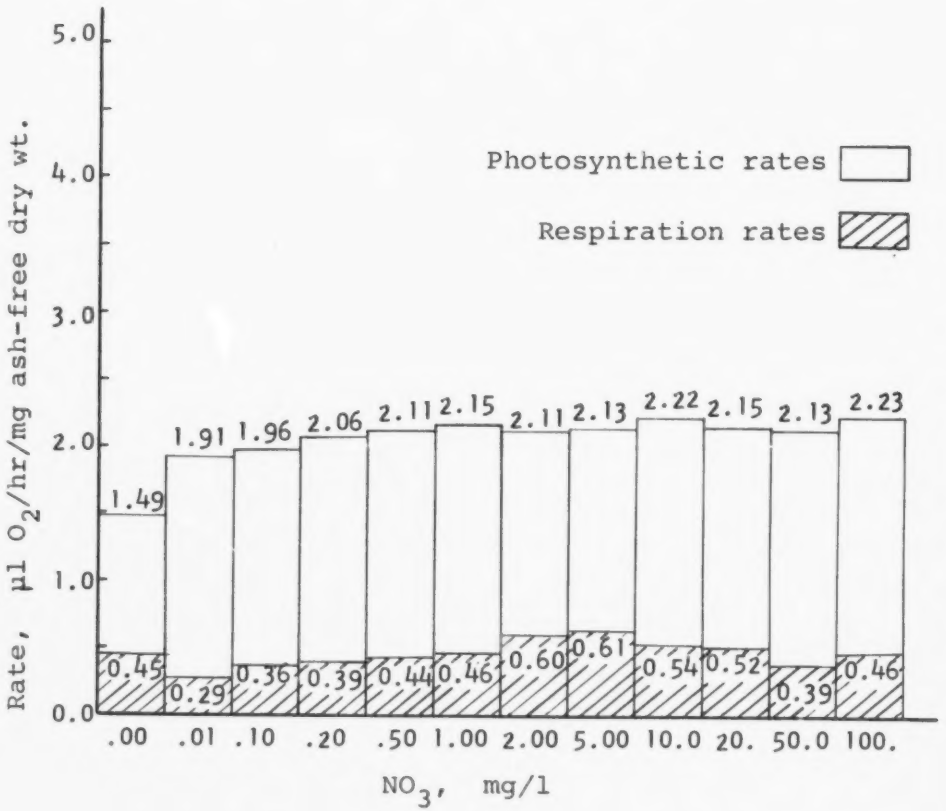

Figure 8. Respiration and photosynthetic rates of Cladophora glomerata in various nitrate concentration without phosphate under laboratory conditions. Each value is the average of 7 measurements.

and then to stay constant, and no decline was observed within the limits of this experiment.

The data obtained in these measurements were also evaluated with a statistical analysis by means of the analysis of variance in both one-way and two-way classifications. ANOVA 1WAY and ANOVA 2WAY of the Tape Storage and Retrieval system on an IBM 360 computer were used to analyse the results of the experiments. The programming of the analysis for Phase 2 is given in Table 14. The outputs of the analysis are not presented in the text, but only the F values are given in Table 15.

Thirteen analyses (Table 15) were made to evaluate the differences among all observed data in Table 11. They were for: 1) total population, 2) total population excluding first row and first column, 3) intersection of first two rows and first two columns, 4) first row, 5) first column, 6) first row excluding first cell, 7) first column excluding first cell, 8) first two cells of first row, 9) first two cells of first column, 10) first three cells of first row, 11) first four cells of first row, 12) second and third cells of first row, and 13) third and fourth cells of first row. Each analysis has been done for both the respiration and photosynthetic rates. These analyses were not made at one time. The latter analyses were found to be necessary by the results of former analyses.

TABLE 14.

PROGRAMMING FOR PHASE 2

```
//PROG2    JOB 'CIVLE T561','SHUNN-DAR LIN',MSGLEVEL=1
//JOBLIB DD DSNAME=SYSI.TSARLIB,DISP=OLD
// EXEC PGM=EXEC
//SYSPRINT DD SYSOUT=A
//SYSIN DD*
DATA RUN
RR + PR OF C. G. IN VAR CONC OF NO3 + PO4 LAB      LIN,S.D.
OPTION CARDDATA
ID NONE NO3 1-5 PO4 6-10 RR 12-15 PR 17-20

GROUP 1=0 WITH PO4 EQUAL   .00
GROUP 2=0 WITH PO4 EQUAL   .01
GROUP 3=0 WITH PO4 EQUAL   .10
GROUP 4=0 WITH PO4 EQUAL   .50
GROUP 5=0 WITH PO4 EQUAL  1.00
GROUP 6=0 WITH PO4 EQUAL  2.00
GROUP 7=0 WITH PO4 EQUAL  5.00
GROUP 11=0 WITH NO3 EQUAL    .00
GROUP 12=0 WITH NO3 EQUAL    .01
GROUP 13=0 WITH NO3 EQUAL    .10
GROUP 14=0 WITH NO3 EQUAL    .20
GROUP 15=0 WITH NO3 EQUAL    .50
GROUP 16=0 WITH NO3 EQUAL   1.00
GROUP 17=0 WITH NO3 EQUAL   2.00
GROUP 18=0 WITH NO3 EQUAL   5.00
GROUP 19=0 WITH NO3 EQUAL  10.00
GROUP 20=0 WITH NO3 EQUAL  20.00
GROUP 21=0 WITH NO3 EQUAL  50.00
GROUP 22=0 WITH NO3 EQUAL  100.0
GROUP 31=1 WITH NO3 EQUAL  .00
GROUP 32=2 WITH NO3 EQUAL  .00
GROUP 33=3 WITH NO3 EQUAL  .00
GROUP 34=4 WITH NO3 EQUAL  .00
GROUP 35=5 WITH NO3 EQUAL  .00
GROUP 36=6 WITH NO3 EQUAL  .00
GROUP 37=7 WITH NO3 EQUAL  .00
GROUP 41=11 WITH PO4 EQUAL  .00
GROUP 42=12 WITH PO4 EQUAL  .00
GROUP 43=13 WITH PO4 EQUAL  .00
GROUP 44=14 WITH PO4 EQUAL  .00
GROUP 45=15 WITH PO4 EQUAL  .00
GROUP 46=16 WITH PO4 EQUAL  .00
GROUP 47=17 WITH PO4 EQUAL  .00
```

TABLE 14.

PROGRAMMING FOR PHASE 2 (CONC.)

```
GROUP 48=18 WITH PO4 EQUAL  .00
GROUP 49=19 WITH PO4 EQUAL  .00
GROUP 50=20 WITH PO4 EQUAL  .00
GROUP 51=21 WITH PO4 EQUAL  .00
GROUP 52=22 WITH PO4 EQUAL  .00
0    ANOVA 2WAY RR 1 2 3 4 5 6 7
     11 12 13 14 15 16 17 18 19 20 21 22
0    ANOVA 2WAY PR 1 2 3 4 5 6 7
     11 12 13 14 15 16 17 18 19 20 21 22
0    ANOVA 2WAY PR 2 3 4 5 6 7
     12 13 14 15 16 17 18 19 20 21 22
0    ANOVA 2WAY PR 2 3 4 5 6 7
     12 13 14 15 16 17 18 19 20 21 22
0    ANOVA 2WAY PR 1 2
     11 12
0    ANOVA 2WAY PR 1 2
     11 12
0    ANOVA 1WAY PR 31 32 33 34 35 36 37
0    ANOVA 1WAY PR 31 32 33 34 35 36 37
0    ANOVA 1WAY PR 41 42 43 44 45 46 47 48 49 50 51 52
0    ANOVA 1WAY PR 41 42 43 44 45 46 47 48 49 50 51 52
0    ANOVA 1WAY PR 32 33 34 35 36 37
0    ANOVA 1WAY PR 32 33 34 35 36 37
0    ANOVA 1WAY PR 42 43 44 45 46 47 48 49 50 51 52
0    ANOVA 1WAY PR 42 43 44 45 46 47 48 49 50 51 52
0    ANOVA 1WAY PR 31 32
0    ANOVA 1WAY PR 31 32
0    ANOVA 1WAY PR 41 42
0    ANOVA 1WAY PR 41 42
0    ANOVA 1WAY PR 31 32 33
0    ANOVA 1WAY PR 31 32 33
0    ANOVA 1WAY PR 31 32 33 34
0    ANOVA 1WAY PR 31 32 33 34
0    ANOVA 1WAY PR 32 33
0    ANOVA 1WAY PR 32 33
0    ANOVA 1WAY PR 33 34
0    ANOVA 1WAY PR 33 34
END
{ .00  .00  .37 1.21 }
                      } 588 data cards
END
/*
```

TABLE 15

RESULTS OF F DISTRIBUTION FOR THE ANALYSIS OF VARIANCE IN TABLE 11.

Analysis No.	Groups to be Analyzed	Classification	For Rate of	Effect	F*	$F_{.95}(m,n)^{\dagger}$	$F_{.99}(m,n)^{\dagger\dagger}$	Difference
1	Total population	2 way	Respiration	Columns	83.29	2.10	2.80	Significant
				Rows	12.90	1.79	2.25	"
			Photosynthesis	Columns	107.42	2.10	2.80	"
				Rows	28.94	1.79	2.25	"
2	Total population excluding first row and first column	2 way	Respiration	Columns	37.63	2.21	3.02	"
				Rows	11.15	1.83	2.32	"
			Photosynthesis	Columns	70.06	2.21	3.02	"
				Rows	21.41	1.83	2.32	"
3	Intersection of first two columns and first two rows	2 way	Respiration	Columns	17.80	4.24	7.77	"
				Rows	1.30	4.24	7.77	No
			Photosynthesis	Columns	9.83	4.24	7.77	Significant
				Rows	15.24	4.24	7.77	"

* = Computed values

† and †† = From Appendices B-1 and B-2, respectively. m=k-1. k=No. of rows or columns. n=degree of freedom for residual or within.

TABLE 15

RESULTS OF F DISTRIBUTION FOR THE ANALYSIS OF VARIANCE IN TABLE 11 (Continued).

Analysis No.	Groups to be Analyzed	Classification	For Rate of	Effect	F*	$F_{.95}(m,n)^{\dagger}$	$F_{.99}(m,n)^{\dagger\dagger}$	Difference
4	First row	1 way	Respiration		4.22	2.33	3.28	Significant
			Photosynthesis		6.16	2.33	3.28	"
5	First column	1 way	Respiration		3.73	2.00	2.53	"
			Photosynthesis		2.82	2.00	2.53	"
6	First row excluding first cell	1 way	Respiration		2.01	2.49	3.60	No
			Photosynthesis		2.83	2.49	3.60	Either
7	First column excluding first cell	1 way	Respiration		4.01	1.98	2.61	Significant
			Photosynthesis		0.62	1.98	2.61	No
8	First two cells of first column	1 way	Respiration		10.30	4.75	9.33	Significant
			Photosynthesis		29.74	4.75	9.33	"

* = Computed values

† and †† = From Appendices B-1 and B-2, respectively. m=k-1. k=No. of rows or columns. n=degree of freedom for residual or for within.

TABLE 15

RESULTS OF F DISTRIBUTION FOR THE ANALYSIS OF VARIANCE IN TABLE 11 (Concluded).

Analysis No.	Groups to be Analyzed	Classification	For Rate of	Effect	F*	$F_{.95}(m,n)^{\dagger}$	$F_{.99}(m,n)^{\dagger\dagger}$	Difference
9	First two cells of first row	1 way	Respiration		2.74	4.75	9.33	No
			Photosynthesis		5.12	4.75	9.33	Either
10	First three cells of first row	1 way	Respiration		2.73	3.55	6.01	No
			Photosynthesis		4.35	3.55	6.01	Either
11	First four cells of first row	1 way	Respiration		7.59	3.01	4.72	Significant
			Photosynthesis		4.16	3.01	4.72	Either
12	Second and third cells of first row	1 way	Respiration		.26	4.75	9.33	No
			Photosynthesis		1.40	4.75	9.33	No
13	Third and fourth cells of first row	1 way	Respiration		6.41	4.75	9.33	Either
			Photosynthesis		.03	4.75	9.33	No

* = Computed values

† and †† = From Appendices B-1 and B-2, respectively. m=k-1. k=No. of rows or columns. n=degree of freedom for residual or for within.

Table 15 indicates the computed F values as well as the critical region of F-distribution (Dixon and Massey, 1957) with the level of significance equal to 0.05 and 0.01. The tests for differences among means are also shown in the last column of Table 15. The method of determination for differences among means is the same as that used in Phase 1. Those procedures have been discussed in detail in Phase 1, therefore the tests for hypotheses are not given in detail in this section. Three terms were used to express the differences of means. They were Significant, No, and Either. "Significant" and "No" meant there were significant difference with a probability level of 0.01 and no difference, respectively, among means. The term "Either"meant there was significant difference with a probability level equal to 0.05.

It was found (Table 15) that there was no difference 1) the respiration rates in Analysis 3 (NO_3-PO_4 = 0.00-0.00, 0.00-0.01, 0.01-0.00, and 0.01-0.01) in rows effect; 2) the respiration rates in Analysis 6 (no nitrate, and PO_4=0.01 through 5.00 mg/l); 3) the photosynthetic rates in Analysis 7 (no phosphate, and NO_3=0.01 through 100.00 mg/l); 4) the respiration rates in the medium containing no phosphate and no nitrate and in medium of no nitrate and of 0.01 mg/l phosphate (Analysis 8); 5) the respiration rates in Analysis 10 (no nitrate, and PO_4=0.01, 0.10, and 0.50 mg/l); 6) both the respiration and photosynthetic rates in the nitrate-free media of phosphate 0.01 and 0.10 mg/l (Analysis 12); and 7) the

photosynthetic rate in the nitrate-free media which are 0.10 and 0.50 mg/l of phosphate (Analysis 13). Other analyses showed that there were highly significant differences among means with a level of significance of 0.05.

Other observations:

Throughout the period of the investigation temperature and pH determinations of stream water were made from time to time. Biochemical oxygen demand, coliform organisms, total hardness, and dissolved oxygen content of Butternut Creek water were also determined occasionally. These are recorded in Table 16. The sampling time was around 8:30 to 9:00 a.m.

During the period of investigation from July to December, 1966 the stream water temperature varied from 14° C to 21.5° C, then gradually dropped down to about 4° C. A great fluctuation of air temperature was observed from -3.1° C to 25.5° C. The pH values of the water ranged from 7.52 to 8.25. It was found that the pH was higher when the flow was higher. The dissolved oxygen content of water was close to the saturation value for the corresponding water temperature. Biochemical oxygen demand of the water was low, ranging from 1.0 to 4.0 mg/l. Two measurements of coliform bacteria were 5,800 and 13,000 per 100 ml.

TABLE 16

ANALYTICAL RESULTS OF BUTTERNUT CREEK WATER* (1966)

Date	Temperature, °C		pH
	Air	Water	
7/1	23.5	14.5	7.52
7/5	23.5	14.1	7.63
7/11	24.0	16.0	
7/18	24.0	14.5	
7/21	16.2	17.5	
7/25	25.5	17.6	
8/1	23.8	14.5	
8/8	21.0	16.0	
8/12	21.2	16.5	
8/16	23.2	16.5	
8/23†	21.0	21.5	
8/25	16.5	17.2	8.05
8/29‡	20.8	16.0	7.81

* Sampling time was around 8:30 to 9:00 a.m.

† Rain

‡ $D.O.$=8.92 mg/l, Total hardness = 200 mg/l as $CaCo_3$

TABLE 16

ANALYTICAL RESULTS OF BUTTERNUT CREEK WATER (1966).(Concluded)

Date	Temperature, °C		pH	DO in mg/l	BOD in mg/l	MPN per 100 ml
	Air	Water				
9/2	22.2	15.8	8.05			
9/6	16.8	18.8	7.95			
9/8	16.2	16.0	7.91			
9/12	13.2	14.2	7.90			
9/15	12.5	14.3	7.85			
9/19	14.2	13.5	7.60			
9/22	15.0	14.0	7.62			
9/27	8.5	14.2	7.58			
9/29	14.7	13.2	7.77			
10/10	16.6	14.0	8.11			
10/19	17.0	15.5	7.75			
10/25	3.3	9.5	7.78	11.2	1.05	13,000
11/1	10.6	9.2	7.88			
11/7	1.0	8.0	8.28			
11/14	3.5	8.2	7.98	10.8	4.40	
11/21	-3.1	5.2	8.21			
11/22	9.2	6.4	8.18	11.6	3.60	
12/1	First snow					
12/5	1.6	3.9	8.05	12.4	4.40	5,800
12/15	0.4	4.4	7.95	11.8	4.00	

Phase 3. Respiration and Photosynthetic Rates of Cladophora glomerata under Natural Conditions.

In situ respiration and photosynthetic rates were measured at Butternut Creek sampling station once each week from May 10 through June 14, 1967 by means of determinations of oxygen comsumption or production. The results of the in situ metabolic rates of Cladophora glomerata as well as some chemical and physical analyses of Butternut Creek water are presented in Table 17. In Table 17, temperature and the intensity of light are recorded by minimum and maximum readings during each test period.

In the course of this study, the water temperature increased from 8.5 to 19.8° C. The intensity of light at bottle was from 1250 to 6600 f-c. The light transmission ranged from 86 to 94 per cent. The pH values were between 7.82 and 8.05. Only four measurements were made for nitrite and nitrate concentration. The nitrite content of the water was low — from 0.006 to 0.017 mg/l. The nitrate values ranged from 0.405 to 0.592 mg/l with an average of 0.514 mg/l. The ortho-phosphate concentrations of the creek water varied from a low of 0.05 mg/l to a high of 0.21 mg/l, with an average of 0.13 mg/l.

The ash-free dry weight: total Cladophora dry weignt ratios are recorded in percentage in Table 17. Each value is the average of 16 measurements. It ranged from 75 to 82 per cent. Table 17 gives the photosynthetic rate of Cladophora

glomerata at Butternut Creek study site during May and June 1967, which varied from a low of 13.50 on May 10 to a high of 21.76 µl O_2/hr/mg ash-free dry wt. on June 14. With the exception of the low on May 31, there was a weekly increase in photosynthetic activity. The respiration rates of the algae at the study site ranged from a low of 1.92 on May 10 to a high of 3.70 µl O_2/hr/mg ash-free dry wt. on June 14. The same pattern of an increase in weekly respiration rates was found. The ratio of photosynthetic rate to respiration rate was between 5.9 and 7.2 which is not given in Table 17.

Figure 9 shows some observed results at the study site. The relationship between the in situ photosynthetic rate of Cladophora glomerata and water temperature is given in Figure 10.

Table 18 (N.Y. State Dept. of Health, 1957) shows some biological, chemical, and physical tests of Butternut Creek water at the sampling station of this study. These results can be used for reference.

TABLE 17

RESULTS OF IN SITU STUDY AT BUTTERNUT CREEK SAMPLING STATION, JAMESVILLE, NEW YORK (1967).

Date	Temperature, °C		Light Intensity, f-c		Weather	Butternut Creek water, mg/l				Rates†		Ash-free
	Air	Water	at Surface	at Bottle		pH	NO_2^-	NO_3^-	PO_4^{---}	Res.	Phot.	Tot.Wt., %
5/10	9.5-10.6	8.5-8.9	1500-4000	1250-3500	Cloudy	8.02	-	-	.21	1.92	13.56	80.8
5/17	20.8-22.3	11.7-12.5	1900-6600	1650-6000	Sunny	8.05	-	-	.07	2.44	15.90	81.9
5/24	19.4-27.8	13.4-15.5	2500-7200	2200-6600	Sunny	7.95	.011	.484	.11	2.80	18.05	77.9
5/31	11.7-17.0	11.2-13.3	3000-7000	2800-6500	Sunny	7.91	.006	.592	.15	2.61	17.42	78.3
6/7	22.1-23.2	16.9-18.5	2800-7100	2400-6300	Sunny	7.82	.009	.576	.05	2.85	20.22	75.3
6/14	21.9-23.6	19.0-19.8	1500-4200	1300-3700	Cloudy	7.98	.017	.405	.21	3.70	21.76	81.7

† Metabolic rates of Cladophora glomerata, µl O_2/hr/mg ash-free dry wt.

T = Water temperature
% = The ash-free dry wt:total dry wt ratio
R = Respiration rate of Cladophora glomerata
P = Photosynthetic rate of Cladophora glomerata

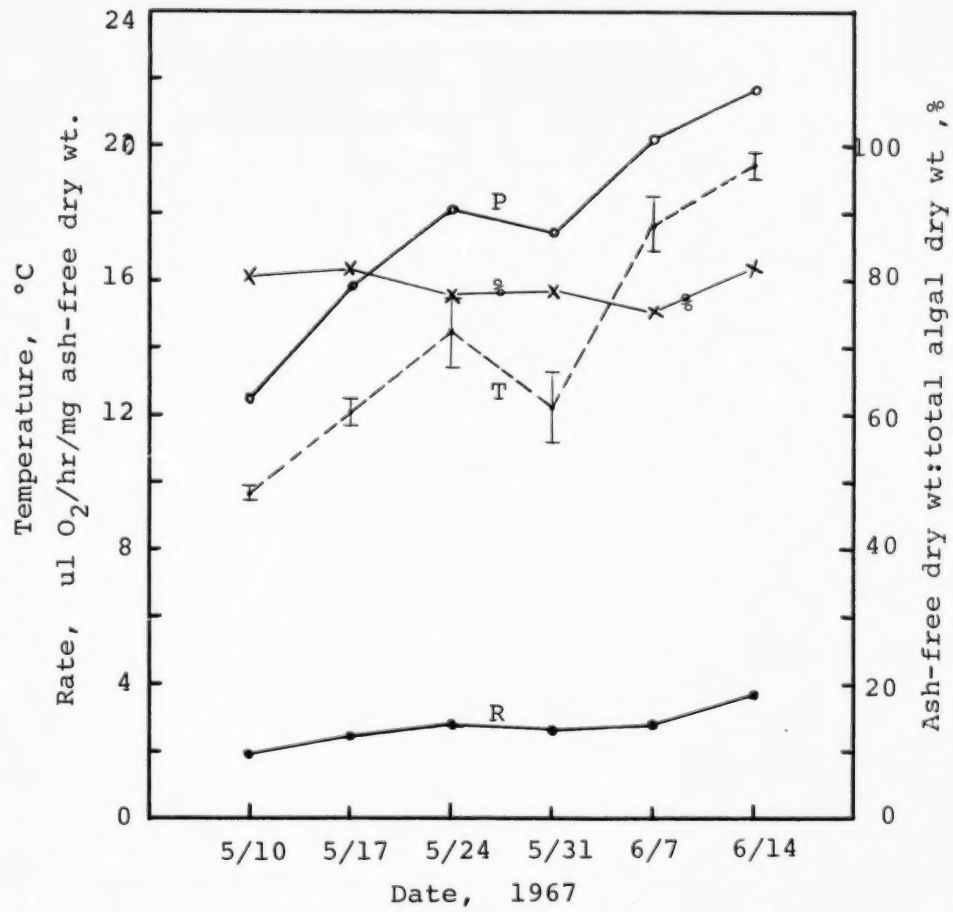

Figure 9. Respiration and photosynthetic rates of Cladophora glomerata, the ash-free dry wt:total dry wt ratio, and water temperature at the study site.

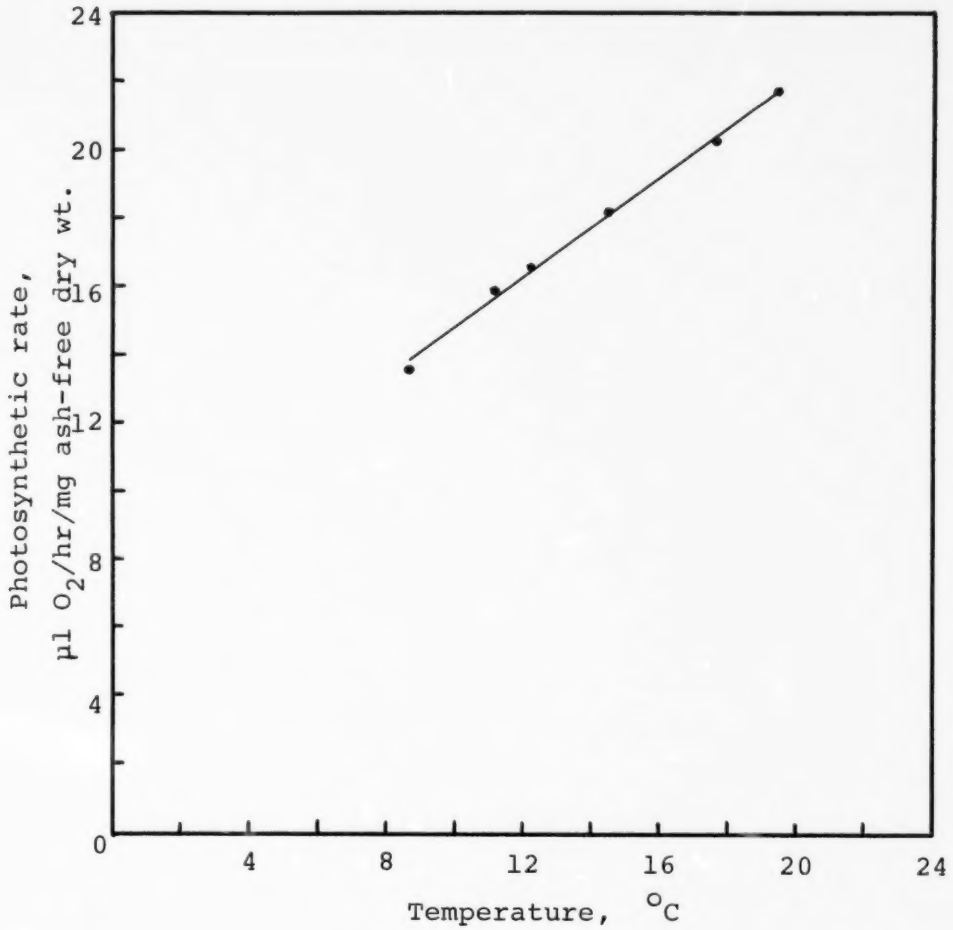

Figure 10. Relation between in situ photosynthetic rate of Cladophora glomerata and water temperature.

TABLE 18

ANALYTICAL RESULTS OF BUTTERNUT CREEK WATER

At Ont. 66-11-P26-37-6(13.9) (N.Y. State Dept. of Health, 1957)

Type of Test	Item	Date and Time	
		8/13/56,-	9/24/56,11:00
Appearance of Stream	Color	NaBr-2	NaBr-1
	Odor	0	0
	Turbidity	2	1
	Suspended matter	1	1
Examination of Sample	Color, ppm	10	-
	Odor	M-2	E-2
	Turbidity, ppm	5	5
	Suspended matter	2	1
	Temperature, ° C.	19	16
	pH	8.2	8.2
	Carbon dioxide, ppm	0.0	0.0
	Dissolved oxygen,ppm	9.0	10.0
	% saturation	96.3	100.5
	B.O.D. -5 Day, ppm	0.8	2.4
	Chlorides, ppm	12.0	6.0
	Alkalinity, ppm	187.0	157.0
	Hardness, ppm	380.0	248.0
	Coliforms, M.P.N. per 100 ml.	9300	110000

V. DISCUSSION

Phase 1. Respiration and Photosynthetic Rates of *Cladophora fracta* under Laboratory Conditions.

In the beginning of this investigation, fragments of *Cladophora fracta* ranging in size from five to 100 cells were used to investigate respiration and photosynthetic rates of *Cladophora fracta*, but this attempt failed. It was found that the rates could not be expressed by µl O_2/hr/cell, because this unit of measurement was too small. Therefore, a small filament of three to six mg of the algae was used for each test.

The metabolic rates of *Cladophora fracta* in a given medium were considerably greater in the flask oscillated at 120 strokes per minute than in that not shaken (F-test). It is thought the reason for this is that nutrients more readily contact the algal cell. These results would indicate that water movement is required for the growth of *Cladophora*. In this study, no attempt was made to determine the optimum shaking speed under laboratory conditions. However, in Lake Ontario where *Cladophora fracta* occurred, the water current varied from zero to 25 feet per minute (OWRC, 1959).

Phosphorus is one of the major nutrient elements required for normal growth of algae. As in all living organisms

compounds containing phosphorus play important roles in nearly all phases of metabolism, particularly in energy transformation reactions. Since phosphorus plays an important role in photosynthesis, the low respiration and photosynthetic rates of Cladophora fracta in the medium containing no phosphorus were expected. The rates in medium M-3 which contained no phosphate and no potassium were the lowest ones (Figure 6). It was found that the respiration and photosynthetic rates of Cladophora fracta in the medium M-3 were approximately two third and one half, respectively, of that in the medium M-1 which contained 55 mg/l of phosphate. These results indicated that the phosphorus is a limiting factor for the growth of the algae.

Maloney (1966) has found that the alga Chlorella pyrenoidosa Chick is able to store phosphorus in excess of its normal metabolic requirements, and uses this reserve for growth in time of phosphorus deficiency. The photosynthesis of Cladophora fracta in the medium M-3 is presumed to be a result of previously stored phosphorus.

The second lowest metabolic rate of Cladophora fracta was observed when the alga was in a nitrogen-free medium; M-2. In comparison with medium M-1 which contained 340 mg/l of nitrate, the rates of photosynthesis and respiration of Cladophora dropped about one-third (Figure 6). Because of

the necessity for nitrogen in all protein formation, a lack of it necessarily causes poor plant development. As nitrogen deficiency develops, the amount of chlorophyll in the cells decreases (Syrett, 1962).

Unexpectedly high rates of respiration and photosynthesis of Cladophora fracta were found in medium M-5 (Figure 6). The rates were higher in medium M-5 than in medium M-1, although the only difference was that medium M-5 contained no calcium chloride. It must now be determined whether the calcium or the chloride inhibited the photosynthesis. The medium M-1 contained 36 mg/l of calcium and 64 mg/l of chloride,neither of which was in the medium M-5. Chloride concentration in the medium M-5 was negligible, because it only had 2.0 mg/l of $M_nCl_2 \cdot 4H_2O$.

In general, green algae require only microquantities of calcium or do not require any calcium. Chloride has been reported as a " coenzyme " of photosynthesis specifically concerned with oxygen evolution. Chlorine and manganese are two catalytic elements to be specifically considered in oxygen evolution during photosynthesis (Eyster, 1964). In this investigation, it has been found that 100 mg/l of $CaCl_2$ inhibited the photosynthesis of Cladophora fracta.Cladophora grow in waters which contain 23.4 to 42.2 mg/l of calcium and 6.6 to 22.5 mg/l of chloride (Table 19). The chloride concentration (64 mg/l) of the medium M-5 was about three times higher than that of Lake Ontario water where Cladophora

TABLE 19

CALCIUM AND CHLORIDE CONCENTRATION AND *CLADOPHORA* IN NATURAL WATERS

Water Course	Ca^{++}, mg/l	Cl^{-}, mg/l	*Cladophora*	Reference
St. Lawrence R. Ogdagsburg,N.H.	31.	7.7		Coker (1954
Butternut Creek,Jamesville,N.Y.	42.2	10.8	p*	Lin (1967)
Lake Ontario, Oswego, N.Y.	41.8	22.5	p	" "
Lake Ontario	38.8	-	p	Kramer (1962)
Lake Erie	23.4	6.6	p	Reid (1961)
Lake Michigan	33.	7.1	p	Risley *et al.* (1965)
Indiana Harbor, Ind.	-	7.1	p	" (1966)
Calumet Harbor, Ill.	-	7.9	p	" "
Mississippi R.Minneapolis,Minn.	40.	1.6		Coker (1954)
Soap Lake, Wash.	20.6	5467.		" "
Bunny Lake, Calif.	0.7	0.2		Reid (1961)
Tennessee R. Gilbertsville,Ala.	19.0	3.		Coker (1954)
Colorado R. Grand Canyon, Ariz.	94.0	92.		" "
Silver Spring, Fla.	68.0	7.8		Reid (1961)
Savannah River, Ga.	4.3	2.4		Coker (1954)
Rain	0.1-10	0.5	-	Reid

* Present

occur. Presumably, too high a chloride concentration inhibited the metabolic rates of Cladophora fracta.

Magnesium and sulfur are considered as macronutrients for the growth of green algae. Sulfur is a part of certain plant proteins. It is regarded as playing a role in respiration and in some plants may affect the photosynthesis. Magnesium is a part of the chlorophyll molecule and as such affects the process of photosynthesis. The respiration and photosynthetic rates of Cladophora fracta in medium M-4 lacking magnesium and sulfur were about 75 and 85 per cent of those in medium M-1, with and without oscillation, respectively.

The essential need by plants for iron has been known for more than a century. Iron deficiency results in a severe chlorosis of the leaves, because iron is not readily transported within the plant. The function of iron is associated with numerous iron-containing enzymes, e.g. peroxidase, catalase, cytochrome c, cytochrome oxidase, cytochrome f, cytochrome b_6, and photosynthetic pyridine nucleotide reductase (Eyster, 1964). In other words, iron is necessary for the formation of chlorophyll even though it does not become a part of the chlorophyll itself. It seems to act as a catalyst in the formation of chlorophyll.

Manganese is one of the key elements in photosynthesis. The requirement is greater for autotrophic growth than for heterotrophic growth (Eyster, 1964). In this investigation, results (Figure 6) have also supported that iron and

manganese play important roles in the algal photosynthesis. The rates of photosynthesis of Cladophora were decreased approximately 25 per cent in the medium M-6 (no iron) and 20 per cent in the medium M-7 (no manganese) from those in the medium M-1. In flasks oscillated at 120 strokes per minute, the respiration rates of the algae in both media M-1 and M-7 were the same. Thus, it is concluded that manganese had no effects on the respiratory process, but iron seemed to play a part in some respiratory processes.

Phase 2. Respiration and photosynthetic Rates of Cladophora glomerata under Laboratory Conditions.

The media used in this study have been modified from the media used in Phase 1. Sodium nitrate instead of nitric acid was used as the source of nitrogen. This modification made it easier to adjust the pH value of the medium. The concentration of calcium chloride was only one-fourth that used in Phase 1. This calcium chloride quantity was the same used by Maloney (1966).

Many investigations have shown that nitrogen and phosphorus are both essential for the growth of algae. Nitrogen is usually used in combined form such as a nitrate. Phosphate is actually incorporated in certain essential plant proteins. It is also involved in reactions where energy exchanges are important, for example, in the form of ATP. Research also

has shown that limitation in amounts of these elements is usually the factor that controls the rate of algal growth.

The purposes of this series of studies were to determine: 1) nitrate and phosphate effects on Cladophora glomerata, 2) optimum concentration of nitrate and phosphate required by the algae, 3) whether there is a interaction effect between nitrate concentration and phosphate concentration. From the statistical analysis (Table 15) of the data, the following indications are obtained:

It may be seen from Analyses 1, 2, and 3 of Table 15 that the differences of metabolic rates of Cladophora glomerata in different combinations of nitrate and phosphate concentrations were highly significant for both columns and rows effects, except the rows effect of respiration rates in the Analysis 3. This exception may be a result of the respiration rate in medium containing 0.01 mg/l of nitrate and no phosphate (0.29 μl O_2/hr/mg ash-free dry wt.) being much smaller than in the medium containing no nitrate and phosphate (0.45 μl O_2/hr/mg ash-free dry wt.). These highly significant differences in the means of the metabolic rates of the algae indicated that the rates increased as either the nitrate concentration or phosphate concentration increased. The photosynthetic rate increased significantly at the 0.01 probability level as either nitrate concentration or phosphate concentration was increased from 0.00 to 0.01 mg/l (Table 13 and Analysis 3 of Table 15).

Streams and rivers containing phosphate concentrations less than 0.01 mg/l are rare. It is concluded that a lotic environment with more than 0.01 mg/l of phosphate might stimulate the Cladophora growth, but this quantity is not an optimum concentration. This concentration level was about one-third the value obtained by Sawyer (1952). He suggested the critical level of phosphorus for algal growth was somewhere near 0.01 mg/l (approximately 0.03 mg/l of phosphate).

Further analyses were made to evaluate the mean rates of differences under conditions of no phosphate and no nitrate. Analysis 4 in Table 16 indicated that the differences among the respiration or photosynthetic rates of Cladophora glomerata in media in which phosphate concentration ranged from 0.00 to 5.00 mg/l (no nitrate) were significant at the 99 per cent level of confidence. However, no significant differences among the respiration or photosynthetic rates of the algae in various phosphate concentrations from 0.01 to 5.0 mg/l (no nitrate) were found (Analysis 6 of Table 16). Analyses 9 through 13 were made to determine the critical phosphate concentration (no nitrate) of the algae. The highest respiration rates of the algae were observed with phosphate, concentration of 0.5 mg/l.

Under the condition of no phosphate, the respiration rates of the algae were low,then increased and eventually declined as the nitrate concentration ranged from 0.00 to 100.00 mg/l (Figure 8 and Table 12). The highest rate was found in the media containing 2.00 and 5.00 mg/l of nitrate. Without

phosphate in media, Analyses 5, 7, and 8, of Table 15 illustrate that the photosynthetic rate of Cladophora glomerata in media containing nitrate is higher than that in the nitrogen-free medium, but the difference in nitrate concentration did not affect the photosynthetic rates significantly.

The above are merely theoretical considerations, since phosphorus-free or nitrogen-free water is not found in nature. Hutchinson (1957) reported a mean phosphorus value for world lakes to be from 0.011 to 0.038 mg/l. The value of nitrogen for unpolluted lakes is less than 0.4 mg/l and above 1.0 mg/l for polluted lakes. Domestic sewage contains about 20 to 100 mg/l of nitrogen and from ten to 60 mg/l of phosphorus. Feth (1966) reported that the concentrations of nitrogen compounds ranged from 0.0 to 100 mg/l in surface water.

It is apparent from Table 15 and Figure 7 that there is a direct increase between the increase in nitrate and phosphate concentrations. This association is greater between the rates with no phosphate and those at the 0.01 mg/l phosphate or higher concentrations. Turano (1966), however, reported there was no significant change in the photosynthetic rate of Cladophora glomerata in the range of from 0.0 to 0.3 mg/l phosphate.

The respiration rate of the algae tended to be erratic and did not respond to nitrate concentrations with no phosphate. This showed the importance of phosphorus to the metabolic activity of the algae.

There was a slight tendency for the metabolic rates of Cladophora glomerata to be depressed at the highest phosphate and nitrate level. However, no inhibition was observed in high concentrations up to 100.00 mg/l of nitrate and 5.00 mg/l of phosphate. From the results of Tables 12 and 13 the range of phosphate concentrations for the algae was from 0.5 to 5.0 mg/l while the nitrate level ranged from 2.0 to 50.0 mg/l. In low concentrations of both nutrients, such as 0.01 mg/l, the metabolic rates of Cladophora glomerata were significantly increased as compared to the rates observed in the absence of these nutrients.

It was one of the purposes of this study to determine whether there is any effect of the nitrate-to-phosphate ratio upon Cladophora glomerata. The statistical analyses of results by F-test (Detailed calculations are not in the text) indicated that the nitrate-to-phosphate ratios ranged from 5:1 to 20:1. Sawyer (1952) reported that the N:P ratios of 30:1 were optimum for some algae while for others 15-18:1 ratios appeared optimum. Also Gerloff and Skoog (1957) reported that a N:P ratio of 30:1 was optimum for the growth of a particular species of the blue green algae. Abbott (1967), however, found that nitrate and phosphate consumption occurred independently in his microcosm studies.

The pH of the medium was checked periodically during the experiments in Phases 1 and 2. It was found that there was no

significant pH change after two dark and two light periods of the experiments. The pH value of the medium was 7.5, which van den Hoek (1963) and Turano (1966) also reported optimum for the Cladophora.

Phase 3. Respiration and Photosynthetic Rates of Cladophora glomerata under Natural Conditions.

The reasons for studying the respiration and photosynthetic rates of Cladophora glomerata under natural conditions were: first, to measure oxygen consumption and production of Cladophora glomerata in its natural environments and second, to define some of the biological, chemical, and physical characterestics of the Butternut Creek water.

An attempt was made to measure the dissolved oxygen content of the water in the test (BOD) bottle with a Precision Scientific Galvanic Cell Oxygen Analyser. It was found that the oxygen analyser was not possible in this test because the oxygen analyser requires one foot per second flow and water must be mixed for two minutes before reading. Therefore, the oxygen analyser was not used for this study. The Alsterberg azide modification of the Winkler method was used to determine the dissolved oxygen content of the water in the bottles.

A difference was observed between the metabolic rates obtained under laboratory conditions and under natural conditions (Tables 12, 13, and 17). The respiration and photosynthetic rates of Cladophora glomerata measured at the study

site were about three and six times respectively greater than those under laboratory conditions. The question arises here as to why. A possibility is that Cladophora is sensitive to changes in environmental conditions, such as pH, temperature, nutrients, and light, etc. In Phase 2, the laboratory environmental conditions were as follows: pH value of the synthetic medium was 7.5; temperature was 17° C; the flasks were oscillated at 120 strokes per minute; nutrients varied and equipment was Warburg apparatus. Under natural conditions, temperature varied from 8.5 to 19.8° C; pH values ranged from 7.82 to 8.05; the average nutrient value of the creek water was 0.54 mg/l of nitrate and 0.13 mg/l of phosphate; and oxygen was measured by Winkler method. The difference between the natural and laboratory conditions was also the light intensity. The intensity of light under the natural conditions (1,250-6,600 f-c) was about one to five times greater than that under the laboratory conditions (1,400 f-c). The intensity of light was a factor in deviation between two environments. Accoring to Mason (1962), the Cladophora glomerata was found in water which varied between a temperature of zero and 27.2 °C and pH between 7.1 and 10.1. He also reported that Cladophora could exist under a wide range of light intensities and nutrient concentrations.

It would seem from Figure 10 that there is a definite relationship between water temperature and the photosynthetic rate of Cladophora glomerata.A linear relationship was observed

for water temperature from 8.5 to 20° C. In this temperature range the photosynthetic values were from 13.56 to 21.76 µl O_2/hr/mg ash-free dry wt.

The growth of Cladophora glomerata in Butternut Creek was observed from time to time during the two years study period. The algae were found to live throughout the year. The same was reported by Mason (1962). Cladophora begins its active growing period in the late part of April. The filaments are yellow-green in color at this time. Cladophora glomerata occurs about two weeks earlier than Cladophora fracta does in the Great Lakes (Neil and Owen, 1964). Conditions for the growth of Cladophora glomerata seem to be more suitable on the vertical side of rocks than on bottom rocks. During April and May, 1967, a rapid growth and spread of the algae occurred and by the early part of May all the suitable habitat was covered with filaments. The longest filaments measured were 14 inches long and dark green in color. At the end of May the filaments were less firmly attached to the rocky substrates and readily broken by the flow current. Approximately 30 per cent of the algae filaments remained at the study site on May 31, 1967, and they were yellow green in color. Some free floating filaments could be seen in the running water. About 90 per cent of the filaments were washed away by the flow current by June 7, 1967. At this time the ash-free content of the algae was low (Table 17), and in most areas the filaments did not exceed three inches in length.

After the removal of first crop, further growth generally took place. In 1966 the second crop grew from July through December and it appeared to be the same as the first crop. According to Neil and Owen (1966), however, the yield of the second crop is usually less than that of the first crop in the Great Lakes.

VI. CONCLUSIONS

The respiration and photosynthetic rates of Cladophora fracta and Cladophora glomerata have been measured under laboratory conditions. Also, a field study has been made at the sampling site of Butternut Creek, Jamesville, New York. From results reported on these studies the following conclusions may be drawn:

1. The respiration and photosynthetic rates of Cladophora fracta in a given medium were significantly greater in the flask oscillated at 120 strokes per minute than in that with no oscillation.

2. The metabolic rates of Cladophora fracta have been measured in a basal medium (M-1) and in a basal medium lacking one of the following: HNO_3, K_2HPO_4, $Mg\ SO_4$, $CaCl_2$, $FeC_6H_5O_7$, and $MnCl_2$. The highest rates were observed in the basal medium lacking calcium chloride. Whether calcium or chloride inhibited the metabolic rates of Cladophora fracta was not established. Markedly low rates were obtained in media lacking nitrate and phosphate.

3. For small increases of either nitrate or phosphate concentration from 0.00 to 0.01 mg/l, the photosynthetic rate of Cladophora glomerata increased significantly.

4. Under the condition of no phosphate, the respiration rate of Cladophora glomerata was erratic and did not respond

to nitrate concentrations. The photosynthetic rate of the algae was not affected by nitrate concentration varying from 0.01 to 100.00 mg/l. It is concluded that phosphorus is a limiting factor of Cladophora growth.

5. In the nitrate-free media, the metabolic rates of Cladophora were not influenced by the phosphate concentration levels from 0.01 to 5.00 mg/l.

6. In general, the respiration and photosynthetic rates of Cladophora glomerata increased as the combination of nitrate and phosphate doses increased. The optimum concentrations would be from 0.5 to 5.0 mg/l for phosphate and from 2.0 to 50.0 mg/l for nitrate. No inhibition was observed for nitrate concentration up to 100.0 mg/l and phosphate concentration to 5.0 mg/l.

7. The nitrate-to-phosphate ratios for Cladophora glomerata ranged from 5:1 to 20:1.

8. The pH value of the medium was 7.5 and did not change during each experiment.

9. The average in situ respiration and photosynthetic rates of Cladophora glomerata were 2.72 and 17.82 µl O_2/hr/mg ash-free dry wt, respectively. The respiration and photosynthetic rates of Cladophora glomerata measured under natural conditions were about three and six times respectively greater than those under laboratory conditions. The reasons for these great differences of the metabolic rates under two conditions are not clear. Environmental factors, such as the intensity

of light, temperature, pH, nutrient, and quantity of the algae used are all possible causes.

10. A linear relationship existed between the photosynthetic rates of Cladophora glomerata and the water temperature.

11. Cladophora glomerata begins its active growth in Butternut Creek in the spring during the latter part of April, followed by a rapid growth in May. This first crop developed prior to June and the other main crop was in the period from July to December. No difference in quantity was observed between these two main crops. The color of the filaments changes periodically from yellow green to dark green.

Appendix. The Theory of Differential Respirometer

Umbreit et al. (1964) summarized the theory of the differential respirometer for use in measurements of respiration and photosynthethesis. The determination of the system constant is as follows:

Let: R = Reaction vessel.

L = Compensation vessel.

V_g = µl of gas in the reaction-vessel side of the manometer.

V'_g = µl of gas in the compensation-vessel side of the manometer.

V_f = µl of liquid in the reaction vessel (including ceels).

V'_f = µl of liquid in the compensation vessel.

A = Cross-section area of the manometer capillary, in mm^2.

h = Manometer reading in mm.(the difference in height of the manometer liquid in the limbs of the manometer).

P_o = Normal pressure (760 mm. Hg), in mm. of manometer liquid.

P = Atmospheric pressure (corrected), in mm. of manometer liquid.

Δp = Increase in pressure of the confined gas in the reaction vessel.

$\Delta p'$ = Increase in pressure of the confined gas in the compensation vessel.

P = Pressure of water vapor in the gas spaces, in mm. manometer liquid.

T_o = 273° Absolute.

T = Temperature of the gas and liquid in the vessels in absolute degrees.

α = Solubility coefficient of the gas produced or absorbed (µl per µl of liquid).

Initially, the amount of gas in each gas space is:

$$\text{for } R = V_g \frac{273}{T} \frac{P-p}{P_o} \qquad \text{for } L = V'_g \frac{273}{T} \frac{P-p}{P_o}$$

If X amount of gas is evolved in R, the manometer reading will be h. The increase in volume of gas in R is then $\frac{h}{2}A$ and the decrease in volume of gas in L is $\frac{h}{2}A$. But h does not give the value of ΔP of the gas in R because of the compensatory effect of the pressure rise in L. In other words, both pressure and volume increase in R while the pressure increases and the volume decreases in L. Considering these changes, the final volumes of the gas spaces will be:

$$\text{for } R = (V_g + \frac{h}{2}A) \quad (\frac{P + \Delta P - p}{P_o})$$

$$\text{for } L = (V'_g - \frac{h}{2}A) \quad (\frac{P + \Delta P' - p}{P_o})$$

Since the pressure has increased in each vessel, the increase in the amount of gas dissolved in the liquid must be taken into account. It is:

$$\text{for } R = V_f \alpha \frac{\Delta P}{P_o} \qquad \text{for } L = V'_f \alpha \frac{\Delta P'}{P_o}$$

The amount of gas, X, produced in R is equal to the

sum of the final volume of the gas space and the increase in the amount of the gas dissolved in the water minus the original volume of the gas space; or for R,

$$X_r = (V_g + \frac{h}{2}A)\ (\frac{273}{T}\ \frac{P+\Delta P-p}{P_o}) - (V_g \frac{273}{T}\ \frac{P-p}{P_o}) + (V_f \alpha \frac{\Delta P}{P_o})$$

$$\text{and } X_r = V_g \frac{273}{T}\ \frac{\Delta P}{P_o} + \frac{h}{2}A\ \frac{273}{T}\ \frac{P+\Delta P-p}{P_o} + V_f \alpha \frac{\Delta P}{P_o}$$

$$\text{and } X_r = \Delta P\ [\ \frac{V_g \frac{273}{T} + V_f \alpha}{P_o} + \frac{A}{2}\ \frac{273}{T}\ \frac{(P-p)\frac{h}{\Delta P} + h}{P_o}\ ]$$

On the other hand, no gas was produced in L; but the changes in pressure and volume must be accounted for. In the same manner as above,

$$X_l = 0 = \Delta P'\ [\frac{V_g' \frac{273}{T} + V_f' \alpha}{P_o} - \frac{A}{2}\ \frac{273}{T}\ \frac{(P-p)\frac{h}{\Delta P'} + h}{P_o}]$$

$$\text{and } \Delta P' = h\ [\frac{\frac{A}{2}\ \frac{273}{T}\ (P+\Delta P'-p)}{V_g'\ \frac{273}{T} + V_f' \alpha}\ ]$$

As the pressure was originally the same on both sides of the manometer, and since the pressure has increased by ΔP and $\Delta P'$ in R and L respectively, the difference in pressure as measured on the manometer is,

$$P + \Delta P' + h = P + \Delta P$$

$$h = \Delta P - \Delta P'$$

$$\text{or } \Delta P = h + \Delta P' = h + h \left[\frac{\frac{A}{2} \frac{273}{T} (P + \Delta P' - p)}{V_g' \frac{273}{T} + V_f' \alpha} \right]$$

$$\Delta P = h \left[1 + \frac{\frac{A}{2} \frac{273}{T} (P + \Delta P' - p}{V_g' \frac{273}{T} + V_f' \alpha} \right]$$

Therefore,

$$X = h \left[1 + \frac{\frac{A}{2} \frac{273}{T} (P + \Delta P' - p)}{V_g' \frac{273}{T} + V_f' \alpha} \right] \left[\frac{V_g \frac{273}{T} + V_f \alpha}{P_o} + \frac{A}{2} \frac{273}{T} \frac{(P-p) \frac{h}{\Delta P} + h}{P_o} \right]$$

It has been shown by many investigators that (P-p) and $(P + \Delta P' - p)$ can both be replaced by P without introducing a significant error. It is also permissible to omit V_f' in the second term and to omit the last half of the fourth term of above equation. This results in the simplified equation

$$X = h \left[\left(1 + \frac{A}{2} \frac{P_o}{V_g'} \right) \left(\frac{V_g \frac{273}{T} + V_f \alpha}{P_o} + \frac{A}{2} \frac{273}{T} \right) \right]$$

where the terms within the brackets are the "flask constant" of the respirometer. To obtain the volume, X, of the gas evolved at NTP it is necessary only to multiply the value of the constant by the manometer reading, h.

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